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August 28, 2001

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Dear Dr. Barker:

On May 23, 2001, the Second International Symposium on Nonthermal Medical / Biological Treatments Using Electromagnetic Fields and Ionized Gases, or ElectroMed 2001, adjourned until May 2003. The conference hosted a total of 110 scientists from 10 nations including Europe, Asia, and the Americas. Based on the response to the conference and responses of the participants after the conference, ElectroMed 2001 was a scientific success.

On behalf of the Organizing Committee, I would like to express my gratitude to you and to the Air Force Office of Scientific Research for financial and in kind support that you provided. As indicated in our approved budget, funds were used to hire workshop coordinators, for advertising and promotion, for speaker travel and hotel, and for printing the proceedings of the symposium.

I was also especially pleased with the performance of Dr. Tom Johnson. He did an outstanding job in the review of all of the abstracts, and was primarily responsible for the organizing and printing the ElectroMed 2001 Proceedings. During ElectroMed 2001, he chaired a session and did an excellent and detailed job on site to help bring the meeting to fruition.

As a final report for the ElectroMed 2001 Symposium, I submit the Proceeding of the Symposium.

Once again, I express my sincere thanks and the thanks of the many participants who benefited from the unique concepts of ElectroMed symposia. The support from your office was instrumental to bring ElectroMed 2001 to a successful completion.

Sincerely,

Stephen J Beebe, PhD
Chair, ElectroMed 2001
Associate Professor of Pediatrics and Physiological Sciences

ElectroMed 2001

Second International Symposium on
Nonthermal Medical/Biological Treatments
Using Electromagnetic Fields
And Ionized Gases

May 20-23, 2001
Portsmouth Virginia

Symposium Record Abstracts

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Second International Symposium on
Nonthermal Medical/Biological Treatments
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* *In memoriam*

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Introduction

Recent advances in the generation of ultrashort high electrical power pulses have opened new venues in the field of bioelectrics. Electrical pulses with duration down to less than one billionth of a second but at voltages exceeding ten thousand volts allow us to explore and utilize electrical interactions with biological cells without heating the tissue. The high frequency components in the ultrashort pulses have been shown to provide a pathway to the interior of cells. Pulsed, high power microwave and millimeter wave sources allow us to explore and utilize nonlinear processes on the molecular level, with the potential to modify molecular structures, such as DNA, selectively.

A second technological area which has developed rapidly is the generation of cold ionized gases (cold plasmas). Charged particles, radicals, and high-energy photons generated in these ionized gases are effective in changing their chemistry, with applications in bacterial and chemical decontamination.

This is the second international conference of its kind. Supported by the Air Force Office of Scientific Research, the first meeting "ElectroMed99" was very well received by the scientific community. A large number of invited and contributed papers presented at ElectroMed99 were published in a special issue of the *IEEE Transactions on Plasma Science* (February 2000). Like ElectroMed99, the purpose of ElectroMed2001 is to bring together medical professionals, microbiologists, molecular biologists, physicists, and electrical engineers, to exchange their experience in the field of these emerging bioelectrotechnologies. This conference will provide a forum for review and discussion of the progress, problems, and the potential of high frequency, high power electric pulses and cold ionized gases for applications in medicine and as tools for bacterial and chemical decontamination.

**Invited and
Contributed Talks**

What Are the Risks of Microwave Radiation

Kenneth R. Foster

Professor of Bioengineering and Electrical Engineering

Department of Bioengineering

120 Hayden Hall, 3320 Smith Walk

Philadelphia, PA 19104-6392

For many years controversies have existed about possible health effects of microwave radiation, such as emitted by communications systems including cell phones, radar, microwave ovens, and other applications of this part of the electromagnetic spectrum. In this talk I will review the scientific basis of the interaction between microwaves and tissue, focusing on biophysical mechanisms of interaction as they pertain to hazard mechanisms and thresholds for hazardous effects. Finally, I will review emerging evidence related to possible hazards of cell phones, with particular reference to possible links between use of cell phones and brain cancer. Evidence so far fails to demonstrate health risks from microwave energy at levels below international exposure guidelines. However the literature is confusing and inconsistent in many places, and many scientific questions remain open. Remaining scientific uncertainty, and the widespread public concern about possible health risks of microwaves, creates difficult problems in the regulatory arena and call for careful communication between users of the electromagnetic spectrum, government, and the public.

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- K. R. Foster and J. E. Moulder, Mobile Phones and Brain Cancer: An Update. IEEE Spectrum August 2000. Available on-line at <http://www.spectrum.ieee.org/publicfeature/aug00/prad.html>
- K. R. Foster, P. Vecchia, M. H. Repacholi, Science and the precautionary principle. Science 288: 979-980 (2000).

Biographical Sketch:

Kenneth R. Foster (F) is Professor of Bioengineering at the University of Pennsylvania. Since receipt of the Ph.D. in 1971, Dr. Foster has been engaged in studies on the interaction of nonionizing radiation and biological systems, including studies on mechanisms of interaction and bio-

medical applications of radiofrequency and microwave energy. In addition he has written widely about scientific issues related to possible health effects of electromagnetic fields. He serves on a number of committees related to possible health and safety effects of electromagnetic fields, and is a consultant to the World Health Organization and other groups on these issues. He has published approximately 90 technical papers in peer reviewed journals, numerous other articles, and is the author of two books related to technological risk. His latest book, *Judging Science*, was published by MIT Press in April 1997.

He is Fellow of the IEEE, 1997-9 Chair of the IEEE EMBS Committee of Man and Radiation. He has served in numerous roles in IEEE, including AdCom of IEEE EMBS, associate editor of T-BME, and is a former President of the IEEE Society on Social Implications of Technology and Chair of the IEEE EMBS Committee on Man and Radiation (COMAR). His website is <http://www.seas.upenn.edu/~kfoster/kfoster.htm>.

Electromagnetic Initiation of Transcription at Specific DNA Sites

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Reba Goodman, Department of Pathology

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Discussions of transcription mechanisms focus on chemical reactions in signaling pathways and transcription factors, but initiation of transcription by electromagnetic (EM) fields (7) offers an alternative physical approach to mechanism. EM fields accelerate electron transfer reaction rates (2, 4), and recent observations show that DNA can conduct electrons within its base pairs (1, 6, 11). These studies suggest that EM fields may initiate transcription by interacting with moving electrons in DNA (2, 3), i.e., by generating repulsive (Lorentz) forces that cause chain separation. EM field stimulated transcription appears to require nCTCTn sequences, 8 in a 900bp region of the *c-myc* promoter (8), and 3 in a 70bp region of the HSP70 promoter (9, 10). Removing these specific sequences eliminates the EM field response, and introducing them elsewhere leads to an EM field response. To explore the possibility that nCTCTn sequences can generate large repulsive forces between DNA chains, we estimate the forces of repulsion between chains, assuming that electron affinity is a measure of electron density at each base ($A=0.97$, $G=1.51$, $T=0.81$, $C=0.57$) and that the velocity of electrons at each base is inversely related to electron density. When a current flows through the DNA, the electron velocity, v , determines the force, F , for a particular value of EM field. The repulsive force is opposed by the attraction between chains due to H-bonds, for A-T bonds ($\sim 10\text{kcal/mol}$) and C-G bonds ($\sim 15\text{kcal/mol}$). From the balance of forces (repulsion-attraction), we estimate that sites rich in C and T, as in the identified sequences, would be more likely to come apart when repulsive forces are generated by EM fields. These calculations suggest a plausible mechanism for initiation of transcription by EM fields interacting with flowing electrons in DNA. They also provide a rationale for the ability of the specific sequences, in the two promoters studied, to function as EM field response elements. Electron flow could be a factor in other processes in DNA (e.g., chain melting due to Joule heating), so this approach may be helpful in relating DNA structure to function.

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Electromagnetic Fields Accelerate Electron Transfer

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Cytochrome oxidase (1, 2) and Na,K-ATPase (3,4) are accelerated by electromagnetic (EM) fields. EM fields accelerate moving charges and should affect electron transfer reactions, i.e., moving charge interaction (MCI) model. The cytochrome oxidase reaction involves electrons, and estimates of the speed of the charges in Na,K-ATPase suggest that they too are electrons. To further test the MCI model, we have studied the Belousov-Zhabotinski (BZ) reaction (5), an oscillating redox system of malonic acid, Br^- , BrO_3^- and $\text{Fe}^{+2}/\text{Fe}^{+3}$ catalyst that involves 10 component reactions with many feedback loops. Under conditions where the BZ reaction oscillates at 0.03Hz, a 60Hz, 28 μT field accelerates the reaction by 5%, similar to the two enzyme reactions (6). Temperature dependence of the BZ reaction is in the range for chemical reactions, the rate doubling over 10°C. Increasing the BZ reaction rate by raising the temperature results in a decreased acceleration due to EM fields, similar to Na,K-ATPase and cytochrome oxidase, where there is competition between the applied EM field and intrinsic chemical driving forces. The BZ reaction has been useful for elucidating EM mechanisms, since it shows an effect in the absence of an enzyme, a membrane or calcium ions, and the results support the idea that EM fields exert their biological effects through interaction with moving electrons (6). We have suggested that low frequency EM fields initiate transcription in cells, in particular the stress response (7), by accelerating electrons moving within the DNA double helix (8, 9, 10, 11). The velocity of charge movement calculated from our Na,K-ATPase measurements, 10^3 m/s, is similar to ultrafast electron transfer in DNA of 400 m/s (12). At these velocities, the forces at low field strengths affect enzyme reactions, and may be large enough to cause changes in DNA. Additional support for direct interaction with DNA comes from identification of a 900 base pair segment associated with the response to EM fields. When the DNA segment is removed, the response is elimi-

nated, and when it is transfected into a reporter construct, the construct becomes EM field responsive.

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Effects of Electromagnetic Fields on the Central Nervous System

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A review of the literature on the effects of electromagnetic fields shows that exposure at relatively low intensities under certain conditions could affect the nervous system. These include effects on morphology, electrophysiology, neurotransmitter activity, and metabolism. Studies in our laboratory have shown effects on: psychoactive drug actions, cholinergic and endogenous opioid activities. Possible use of electromagnetic fields for treatment of drug abuse and withdrawal will be discussed.

Low-intensity electromagnetic fields have also been shown to cause genetic damage in cells. Possible use of these fields for cancer treatment will also be discussed.

Anticancer Activity by Nonthermal Magnetic Fields

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In previous studies [1, 2, 3] we used different static and ELF Magnetic Fields (MF) exposures to show that an appropriate modulation of these fields (static fields with a superimposition of ELF MF at 50 Hz), with an average intensity higher than 3.59 mT, results in a 40 to 50% inhibition of tumor growth in nude mice bearing a subcutaneous human colon adenocarcinoma induced by WiDr cell injection, when exposed 70 minimum daily for 5 days a week for 4 consecutive weeks. The inhibition of tumor growth was reported together with a decrease in tumor cell mitotic index and proliferative activity. An increase in apoptosis and corresponding reduction of immunoreactive p53 expression was also reported. Gross pathology, hematoclinical/hematological and histological examinations did not show any toxic or abnormal effects. Similarly we also showed that in the same *in vivo* model system, MF treatment, starting 24 hours after WiDr cell inoculation, significantly increases survival time (31%) for the nude mice exposed daily to 5.5 mT magnetic fields.

It has been hypothesized that MF treatment exerts antitumor efficacy through an influence on redox signaling, triggered by free radicals [4].

More recently, new *in vivo* experiments using different tumor engraftments in different animal models have been performed. Preliminary results confirm the antitumor efficacy of the MF treatment.

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Retaining of the Long-Term Potentiation in Hippocampal Slices after High Peak Power Microwave Exposure and Heating

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The long-term potentiation (LTP) is a profound and long-lasting facilitation of neural response after a brief tetanic stimulation. This facilitation develops within minutes and may persist for hours with little or no decay. The LTP has long been thought to play a key role in memory processes and is often employed as a memory model in *in vitro* studies. The goal of the present work was to analyze if exposure to extremely-high peak power microwave pulses (EHPP) and heating may damage already developed LTP.

Methods:

The experiments were performed in sagittal hippocampal slices (350-mm thick) isolated from the brain of 4- to 6-week old male Sprague-Dawley rats. The slices were placed on a matching plate at the end of a waveguide and submerged into an artificial cerebrospinal fluid (ACSF). The exposure setup, dosimetry and thermometry were essentially the same as described earlier (Pakhomov et al., Bioelectromagnetics, 1999, V. 21, 245-254). *Stratum radiatum* area of the slice was stimulated with a bipolar tungsten electrode at 30-sec intervals, and population spikes (PS) in the CA1 area were recorded by a glass microelectrode (2-10 Mohm). Experiments began after a 30- to 90-min stabilization and lasted for 15 min. Each slice was used in a single experiment. Mean PS amplitude during the first 2 min of the experiment was considered baseline (100%) for each slice preparation. Tetanus (2 sec at 50 Hz) was applied at 2 min into the experiment. A 2-min exposure began at 7 min 45 sec, when the PS increase due to the LTP reached its maximum. A model 337X EHPP

transmitter (Applied Systems Engineering, Inc.) produced 1- μ s wide pulses at the peak power of 280-290 kW. At 5% reflection, the peak transmitted power was about 270 kW (1.57 MV/m), and the peak specific absorption rate (SAR) in the center of the slice reached 360 kW/g. Pulse repetition rates and time-average SAR levels were 2 Hz and 0.7 W/g (group 1), 5 Hz and 1.8 W/g (group 2), and 10 Hz and 3.6 W/g (group 3). Irradiation increased the ACSF temperature from the base level of 34°C to 35.5, 37, and 39.7°C, respectively; initial temperature restored within 1 min after the exposure.

Results:

Effects of sham exposure and 3 exposure regimens were tested in 49 experiments. In all the groups, tetanization increased the PS amplitude to 150-160%. In sham exposed preparations, PS remained at this facilitated level until the end of recording. With EHPP irradiation, PS temporarily decreased, and the decrease was proportional to the time-average SAR and heating. With the maximum heating (group 3), PS dropped to its pre-LTP value ($100 \pm 20\%$). However, in all the exposed groups the PS amplitude successfully recovered soon after the restoration of temperature.

Summary:

Transitory PS suppression during the irradiation and briefly after it can reasonably be explained by heating. However, there was no aftereffect of the irradiation or heating on the LTP. As far as the LTP findings can be extrapolated to memory function, one can say that the EHPP and heating did not "erase" or alter the memory record. The study provided no indication of a specific bioeffect of a brief EHPP exposure, even at peak SAR and incident E-field as high as 360 kW/g and 1.57 MV/m.

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Electrotherapy of Wound Healing: Mechanisms of Action on Cell Structures and Function

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Wound healing is a complex and dynamic process in which electrical activity occurs naturally at the wound site. Studies in the past have shown that natural electrical currents, produced in the wounds of invertebrates and mammals, play a major role in promoting wound epithelialization. Although some clinical trials have demonstrated effectiveness of electrical stimulation (ES) in the treatment of abnormal wound healing, consistent success has not been established. Elucidation of the molecular mechanisms that could couple ES-tissue interactions would lead to clearer understanding of electrotherapy. Detailed characterization of the electrochemical signal transduction mechanisms involved in wound healing, and determination of the range of electrical parameters (e.g., frequency, magnitude, and exposure duration) over which exogenous ES can couple to these mechanisms, are likely to be required before rational clinical therapy can be hypothesized and tested.

Several partially characterized cellular responses to ES suggest that ES-induced perturbations of electrochemical signal transduction may be responsible for such effects. These responses are likely to be initiated at the level of the plasma membrane. We have therefore investigated the effects of exogenous ES on the cellular and molecular mechanisms that could mediate physiological responses in wound healing. Such mechanisms would include redistribution of cell surface receptors, reorganization of cytoskeletal structures, induced cell motility, increases in intracellular Ca^{2+} ion concentration ($[\text{Ca}^{2+}]_i$), and protein trafficking. Based on our results using physiologically relevant ES strengths found at the wound site (2 V/cm), an integrated model is proposed in which ES redistributes receptors on the cell surface and that, in turn, affects interactions between cell surface receptors and cytoskeleton. Reorganized cytoskeletal structures in conjunction with asymmetrical distribution and up-regulation of adhesion proteins (e.g., integrins) are likely to induce cell migration. While cell migration to the wound site during early phase of wound healing, for example, is required for normal and proper wound healing. Unlike sig-

nificant and rapid $[Ca^{2+}]_i$ increases induced by large ES strengths (e.g., 10 V/cm), application of physiological ES strengths is shown to cause gradual and modest $[Ca^{2+}]_i$ increases that may complement and perhaps enhance cell migration, and activate Ca^{2+} -dependent signaling mechanisms. Finally, we plan to examine ES-induced cell adhesion and motility in the reconstituted 3-dimensional tissue equivalent gel. Using biodegradable polymer scaffolds for tissue equivalent, ES is expected to regulate the 3-dimensional gel structures and guide cell adhesion and motility. Application of non-invasive ES is envisioned to improve designing and engineering of tissue equivalents which, in turn, leads to beneficial applications in wound healing and artificial grafts.

Modeling of Biological Cells Subjected to High Intensity Electrical Pulses

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Pulsed electric fields are used for a variety of applications including bacterial decontamination of liquids and food, and the elimination of harmful micro-organisms and bio-hazards. The process of cellular electroporation has been linked to the non-thermal killing of microorganisms subjected to strong electric fields. Depending on pulse amplitude and duration, a certain percentage of the bacteria suffer irreversible damage and die. This mechanism is also useful for introducing drugs or genetic material into cells. Here, some of the electric field-induced effects, particularly electroporation of the cellular membrane, have been analyzed based on time dependent numerical models.

Simulation studies of electroporation in response to ultrashort, high-voltage pulses have been carried out based on a coupled scheme involving the Laplace, Nerst-Plank, and Smoluchowski equations. In our model, a pore-radius dependent energy barrier is used for ionic transport. It is shown that a finite time delay exists in pore formation, and leads to a transient overshoot of the transmembrane potential V_{mem} beyond 1.0 volt. Pore re-sealing is shown to consist of an initial fast process, a 10^{-4} second delay, followed by a much slower closing at a time constant of about 10^{-1} second. This establishes a time-window during which the pores are mostly open, and hence, the system is most vulnerable to destruction by a second electric pulse. The existence of such a time window for effective killing by a second pulse is amply supported by experimental data for *Escherichia coli* cells. The time constant for the longer process matches experiments. Finally, calculations of the cell shape in the presence of an externally applied electric field have also been made. Results suggest a dynamic transition to ellipsoidal geometries having higher eccentricity. This can have consequences in terms of variations in the surface area and affect light scattering, the internal polarization, and cellular response to external AC excitations.

Mapping Membrane Potential Perturbations of Chromaffin Cells Exposed to Electric Fields

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The numerous and varied responses of biological systems to electric fields continue to evoke interest in elucidating the fundamental mechanisms of cellular interactions with these fields. One of the established effects of exposure of a biological cell to electric fields is an induced perturbation in membrane potential of the cell. However, a satisfactory explanation of how electric fields couple to cells in more complicated situations, such as when cells are in aggregates, is yet to be investigated. The primary goal of our research was to investigate the mechanisms of interaction of applied electric fields with cells in aggregates of different size and orientation with respect to the field by assessing the resulting spatial variations in membrane potential.

To address this issue, cultured adrenal medullary chromaffin cells were utilized. These cells manufacture, store, and secrete several hormones including epinephrine (adrenaline) and are considered a model of sympathetic neurons. They form aggregates of various sizes that mimic their appearance in the intact adrenal gland. Chromaffin cells were plated on collagen coated glass coverslips mounted on the bottom of a 35 mm Petri dish in which the central portion has been removed and incubated with the voltage sensitive dye Di-8-ANEPPS in a balanced salt solution for 15 minutes at room temperature. Di-8-ANEPPS is a membrane bound dye that responds to changes in membrane potential with a shift in its emission spectra, thus causing a change in fluorescence. The dish of stained cells was placed on the stage of an inverted Nikon microscope equipped with a 100X oil objective and exposed to a uniform electric field of 25 V/cm created between platinum electrodes embedded in a ring of agarose and energized by a DC power supply. Dye calibration (transformation of

changes in fluorescence to membrane potential) was achieved by chemically clamping the cell membrane potential with a valinomycin-mediated $[K^+]$ diffusion potential. Fluorescence images of cells were acquired before and after exposure to an electric field using an intensified CCD camera. Video sequences were stored on a VCR and digitized by a frame grabber. Image processing and analysis were performed using ImageTool software (University of Texas Health Science Center in San Antonio).

When the electric field was applied to single cells, the membrane potential varied as the cosine of the angle between the electric field and the normal to the membrane. This spatial variation in membrane potential is in agreement with that predicted by Laplace's equation. Cathode-localized depolarization and anode-localized hyperpolarization were observed for both orientations of the field. Work is on going to examine the response of chromaffin cell aggregates of different size upon exposure to electric fields. The basic understanding of the cellular responses to electric fields will provide a foundation for the extension to interaction mechanisms of these fields with cells in tissues.

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Advances in Electroporation Therapy

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The efficacy of potentially useful therapeutics is frequently limited by their low intracellular concentrations achievable by conventional drug delivery methods. This presentation will focus on examples of enhancing chemotherapy and gene therapy of cancer by the method of electroporation (EP), also known as "electroporation." EP uses pulses of short duration (micro to milliseconds) and high field strength (100-1500 V/cm) delivered at the site of treatment, to enhance the permeability of cell membranes and thus increase the intracellular delivery of locally or intravenously injected substances. This treatment amplifies the cytotoxicity of certain anticancer drugs up to 5000-fold, and gene expression of therapeutic DNAs several hundred fold. We have extensively investigated electroporation therapy (EPT) of tumors with anticancer drugs in animal models as well as in clinical studies. EPT is administered by intratumoral injection of the drug, followed by inserting needle array electrodes into the tumor and delivering electric field pulses to the tumor tissue. Among about 20 different drugs tested, bleomycin was found to be the most effective drug in combination with EPT. Bleomycin-EPT results in rapid destruction of tumor cells within 48 hrs after treatment. Histological and molecular analyses reveal that cell death is primarily due to apoptosis rather than necrosis. The destroyed tumor tissue generally forms an eschar which eventually falls off after several weeks and leaves healthy tissue at the previous tumor location. Remarkably, normal tissue is only mildly and transiently affected by EPT. Phase I and II clinical studies in the U.S., Canada and Australia have demonstrated effectiveness of EPT with bleomycin in squamous cell head and neck tumors, basal cell carcinoma and melanoma; the treatment was generally well tolerated. In Europe, the MedPulser® device for electroporation has been approved for commercial distribution and is being introduced selectively for head and neck cancer therapy. A system similar to the MedPulser® has been developed for future use in gene therapy. Using this system for the delivery of plasmid DNA into different target tissues (muscle, skin, tumors), we and others have shown that therapeutic

levels of several gene products can be obtained in animals. Retardation of tumor growth or complete tumor regression has been obtained in mice with several gene constructs. EP has also demonstrated exceptional effectiveness in enhancing the immune response to DNA vaccines, including vaccines against malignant tumors, HBV and HIV. This enhanced effectiveness included both the humoral and cellular response in a variety of animals, including non-human primates.

In conclusion, bleomycin-EPT can be considered an effective, well-tolerated method for treating symptomatic recurrences of head and neck cancer, as well as other types of cancer in humans. While EP-mediated delivery of drugs (and DNA) directly into accessible tumors result in local treatment of tumors, DNA may also be delivered into healthy tissue in order to produce anticancer agents which will facilitate the systemic treatment of cancer. In addition to its effectiveness in treating cancer, EPT is a highly attractive alternative to viral and other non-viral DNA delivery methods which may enable effective and safe treatment of many diseases through gene therapy and DNA vaccination.

Nanosecond Pulsed Electric Field (nsPEF) Applications to Human Cells Results in Selective Intracellular Membrane Disruption

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Application of kV/cm microsecond duration electric fields to form transient pores in the surface membranes of cells for insertion of biomolecules is an important biomedical technology. We have used biochemical, morphologic and electron microscopic methods to examine whether kV/cm, nanosecond electric field applications to cells achieves the effects of pore formation and/or disruption of intracellular membranes. Human eosinophils (Eos), cells that contain large intracellular granules whose highly cationic contents are normally membrane-bound were used as targets. Calcein, a strongly anionic fluorescent dye, was used to stain the cytoplasm of the Eos, and under normal conditions, this dye was fully excluded from the intracellular granules because of the granule membrane. nsPEF applications (10-300 ns, 24-180 kV/cm) to the Eos resulted in development of "sparkler" morphology, i.e., intensely fluorescent intracellular granules without loss of cytoplasmic fluorescence in the 10ns-180 kV/cm, 60 ns-53 kV/cm and 300ns-24 kV/cm conditions. With increasing pulse train size, dose-response effects on the percentage of sparkler cells appearing and the numbers of bright granules/sparkler cell were best demonstrated with the 10 ns-180 kV/cm condition: percentage sparkler cells with 0 pulses: $0 \pm 0\%$ (mean \pm SE); 1 pulse: $17 \pm 3\%$; 10 pulses $76 \pm 2\%$ (all $n=10$); 1+, 2+ or 3+ bright granules/sparkler cell: 0 pulses $0 \pm 0\%$, $0 \pm 0\%$ and $0 \pm 0\%$ respectively; 1 pulse $97 \pm 3\%$, $1 \pm 1\%$ and $1 \pm 1\%$; 5 pulses $54 \pm 4\%$, $32 \pm 3\%$ and $14 \pm 3\%$; 10 pulses $6 \pm 2\%$, $5 \pm 2\%$ and $89 \pm 4\%$ (all $n=10$). Electron microscopy confirmed nsPEF induced rupture of granule membranes. These results demonstrate that selected nsPEF conditions can cause progressive disruption of intracellular membranes without disruption of the surface membrane, with the shorter pulse durations showing the largest gradient of effect.

High Intensity, Nanosecond Pulsed Electric Fields (nsPEF) Induce Apoptosis In Vitro and In Vivo and Inhibit Tumor Growth In Vivo

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Electromanipulation of biological cells, such as electroporation, has been studied for years, but the effects of nanosecond pulses on biological cells have not been tested until now. We analyzed the effects of high intensity (300 kV/cm), nanosecond (10-300 nsec) pulsed electric fields (nsPEF) on human and mouse cells *in vitro* and mouse tumors *in vivo*. Compared to electroporation pulses, nsPEF have durations two to six orders of magnitude shorter, electric fields hundred to thousand times greater, and energy densities five to ten-fold lower.

Objectives:

These studies were designed to determine the effects of nsPEF with different pulse durations on membrane pore formation, subcellular structures and functions, and cell survival. We hypothesize that nsPEF have minimal effects on plasma membrane poration, but induce apoptosis, in part, by targeting mitochondria *in vitro*, which provides a means to induce apoptosis and tumor regression *in vivo*.

Methods:

Human HL-60 and Jurkat cells, mouse fibrosarcoma B10.2 cells, and fibrosarcoma tumors *in vivo* were exposed to nsPEF and analyzed for apoptosis markers by flow cytometry, biochemical assays, and immunoblot analysis. Apoptosis markers included intact cell membrane, annexin V-FITC binding, caspase activation, and changes in cell size and density/granularity. Caspase was also analyzed by enzymatic assay and cytochrome *c* was identified by immunoblot analysis. Apoptosis was determined in tumors by a TUNEL-based method and tumor growth was determined by tumor size and weight.

Results:

As pulse duration decreased, transient membrane poration and pore size were also decreased. nsPEF-induced effects at the membrane and on cell function were distinctly different than electroporation. nsPEF induced apoptosis (programmed cell-death) in cells *in vitro*. Changes in cell size and density were caspase-independent, while annexin V-FITC binding was caspase-dependent. nsPEF induced cytochrome *c* release into the cytoplasm, suggesting that nsPEF targeted the mitochondria, well characterized initiators of apoptosis. nsPEF also induced apoptosis in mouse tumors *ex vivo* and *in vivo*, determined by caspase activation and DNA fragmentation, and decreased tumor growth rate and weight compared to controls.

Conclusions:

These studies support the hypothesis that nsPEF bypass the cell membrane and target subcellular structures [1]. Mitochondria appear to "sense" nsPEF and induce apoptosis similar to other agents that induce apoptosis by mitochondrial-dependent mechanisms. Applications for nsPEF include the selective targeting of subcellular structures and selective deletion of aberrant cells or tissues such as tumors without inflammation.

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Schoenbach KH, Beebe SJ, Buescher ES. Intracellular Effect of Ultrashort Electrical Pulses. (in press) Bioelectromagnetics, 2001

Biomedical Application of Electromagnetic Fields for Cytoprotection And Gene Therapy

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Induction of the stress protein, hsp70, by electromagnetic (EM) fields is often used as an indication of the normal reaction of cells to biological hazards; but it should be emphasized that the same stress protein serves to protect against hazards. We have utilized the protective aspect of EM field induced stress proteins to develop two new beneficial medical tools (patents pending).

(1) EM field-induction of hsp70 for use prior to cardiac by-pass surgery. Elevating hsp70, usually done by hyperthermia (high temperatures), protects the myocardium during reperfusion ischemic stress and helps prevent heart attack and stroke. Induction of increased hsp70 with EM fields eliminates patient discomfort associated with hyperthermia. EM fields are non-invasive, penetrate all cells, and have longer-lasting effects than hyperthermia. Significant levels of hsp70 are induced within 30 minutes and remain elevated for more than 3 hours. Unlike hyperthermia, hsp70 levels can be augmented by restimulation for extended surgical procedures.

Results of cytoprotection studies:

The increase in hsp70 levels by EM fields *in vivo* and *in vitro* depends on the field strength. EM field-preconditioning produces a higher survival rate than thermal preconditioning in fertilized dipteran eggs and cultured rodent cardiomyocytes.

(2) Beneficial use of EM fields for gene therapy. We have identified EM field-responsive elements (EMRE) in the DNA sequences of the HSP70 promoter and of the *c-myc* promoter. A non-invasive medical application incorporates the EMREs in promoters for regulating and programming gene activation. For example, an exogenous insulin gene containing one or more EMREs introduced upstream of the gene, can be

simply and safely regulated by the EM fields. The whole operation becomes automatic by having the EM field generating circuit activated by an implanted glucose sensor that responds immediately to changes in pre-set blood glucose levels.

Results of EMRE studies:

Three nCTCTn DNA sequence sites in the HSP70 promoter and eight in the *c-myc* promoter are EM field-responsive (EMRE). Inactivating these sequences, by removal or mutation, renders promoter constructs unresponsive to EM fields. Inserting this sequence in an unresponsive reporter construct, renders the gene EM field-responsive. This innovation in gene therapy provides a non-invasive and precise technique for gene activation.

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Rapid Sterilization of Surfaces using Advanced Photosensitizers and UV light

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A multidisciplinary team of Engineers, Physicists, Chemists, and Microbiologists have developed a UV light activated photosensitizer process that allows rapid disinfection of spores, viruses, and bacteria on surfaces and in aqueous solutions. The photosensitizer/ disinfectant is a hydrogen peroxide based solution containing a proprietary additive and a surfactant. Experiments at the University of Missouri-Columbia in Cooperation with Clean Earth Technologies have shown that either pulsed or Continuous UV light can be utilized to activate the photosensitizer. The UV light, in conjunction with the photosensitizer generates reactive oxygen species including hydroxyl radicals that interact with the nucleic acids and the lipid proteins in the organisms tested. The demonstrated efficacy of using UV in synergism with a photosensitizer has shown that over seven decades of reduction in viable *Bacillus subtilis* spores is possible in under sixty seconds for spore densities approaching 10^9 spores per square centimeter. Additional reductions in time have also been demonstrated with pulsed UV light sources. High dose rate application of light appears to lead to a decrease in the applied dose for disinfection in combination with our proprietary photosensitizer. Scanning electron microscopy has shown that the UV activated photosensitizer may also be responsible for the damage to the spore coat. The experimental results on plastic glass and metal surfaces are discussed along with the projected rate of disinfection which will be possible with commercial systems. The apparent ability of the process to rapidly disinfect surfaces using a nonthermal process may offer advantages in the medical arena, with subsequent decontamination of equipment in the field a near term possibility.

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Directed Killing of Anthrax Spores by Microwave Induced Cavitation Via Specific Binding of Organic Semi-Conductor

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We previously reported that anthrax spores could be damaged by the action of a pulsed microwave induced thermochemiluminescent response (Kiel et al, IEEE Transactions on Plasma Science 28: 161-167, 2000). They were placed into the volume of liquid that experienced the pulsed flashing effect on a bacterial filter. Therefore, the efficacy of such an approach toward decontamination of a surface contaminated with anthrax spores would be very inefficient because we could not always assure that the flashing would occur where the spores were located. To maximize the effect, we needed to attach the organic semi-conductor diazoluminomelanin (DALM) to the surface of the spores. We also needed to confirm that multiple bubble sonoluminescence and the corresponding sonochemistry were occurring on the spores. Serendipitously, we discovered that DALM biosynthesized by JM109 *E. coli* containing the plasmid pIC2ORNR_{1,1} (American Type Culture Collection # 69905) specifically binds to Sterne Strain anthrax spores, but not to other species of *Bacillus*. The chemically synthesized form of DALM does not demonstrate this property. Therefore, the *E. coli* bacteria must generate another molecule attached to the polymer in the synthesis process that conveys the specificity. DALM does bind to magnetite particles non-specifically, and this property is maintained by both the chemically synthesized and biosynthesized DALM. Upon exposure to pulsed microwave radiation (1.25 GHz, pulse rate of 10 Hz, 6 μ s pulses, peak power of 2 MW), the tagged spores demonstrated killing when flashing occurred, but not when it was absent. Examination of the light emissions demonstrated atomic gas spectra typical of air and organic components

associated with the bacteria and DALM. Such spectra are typical of plasma generated by laser induced breakdown and by multiple bubble sonoluminescence. Therefore, specific directing of the binding of organic semi-conductor to target microbes may be a useful approach for selective pulsed microwave decontamination of a contaminated surface. This work was sponsored in part by the U. S. Air Force Office of Scientific Research, the Joint Services Technical Base Program in Chemical and Biological Defense, and the U. S. Army Medical Research and Material Command under Contract DAMD 17-94-C-4069. The work presented here is the opinion of the authors and does not represent official opinions or policies of the United States Air Force, Army, or any other agency of the Federal government of the United States of America.

The Influence of Pulsed Electric Field Profile on the Inactivation of Micro-organisms

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In recent years, there has been increasing interest shown in the potential use of Pulsed Electric Fields (PEF) for the inactivation of foodborne pathogenic micro-organisms. The specific mechanisms by which PEF is able to destroy liquid borne microorganism has still not been fully identified, although interaction with the cell membrane, and enhanced electroporation, are considered to be involved. It is well known that the electric field intensity and the pulse duration are parameters that can be altered to vary the degree of PEF effectiveness. Pulse durations of 1-20ms and field intensities of ~30kV/cm are generally considered to be of greatest interest. It has also been reported, that the use of a bi-phase pulse, as opposed to a uni-directional pulse, leads to greater level of inactivation. Once again, the mechanisms responsible for this reported increased inactivation have not been elucidated, but it is reasonable to suggest that this may be associated with a greater degree of PEF interaction with the cell membrane.

In order to investigate the influence of the nature of the applied PEF profile on bacterial inactivation, a parallel series of experiments has been undertaken. *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus* were cultured overnight in Typtone Soya Broth and then resuspended in 0.1% peptone prior to PEF exposure. After treatment, survivors were enumerated by dilution plate counting or spiral plate counting techniques. The results compare the effect of the electric field pulse profile on the inactivation of the test bacteria. A 2 μ s uni-directional pulse has been compared with a 2 μ s bi-directional pulse (ie 1 μ s positive -1 μ s negative) in order to identify the significant of the pulse profile. For both pulse profiles, the magnitude of the electric field intensity was identical, as was the total number of pulse applied. In this way, the total energy delivered by each of the PEF systems was identical and the significance of PEF profile should be evident.

Biological and Chemical Decontamination Using Near Atmospheric Pressure Plasma Discharges

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The objective of Atmospheric Pressure Plasma (APP) technology for Chem/Bio Warfare (CBW) Decontamination is to detoxify a surface film of CBW agents using the reactive stream generated by a plasma composed of innocuous gases, such as helium and oxygen. The reactive species, typically atomic oxygen and metastable, molecular oxygen, are generated by passing the feedgas through an ionized gas where it becomes chemically activated through collisions with energetic electrons. Because the reactive species generated by the plasma are short-lived, if they do not react with their target, they quickly deactivate back to relatively innocuous gases posing little or no operator safety or environmental concern. APP decontamination fills a vital niche amongst the various decon methods because, unlike traditional decon methods, APP is dry and nondestructive to sensitive equipment, such as electronics, and irreplaceable objects, such as national treasures. This would provide a fast and portable means of restoration of contaminated items for which the only current option is ultimate disposal. These devices would rely heavily on the novel Atmospheric Pressure Plasma Jet (APPJ) technology which has been developed at Los Alamos National Lab (LANL) over the past six years and was a winner of a 1999 R&D 100 Award.

The APPJ is a unique, non-thermal, glow-discharge plasma operating at atmospheric pressure. The discharge uses a high-flow feedgas consisting primarily of an inert carrier gas, such as He, and a small amount of an additive to be activated, such as O₂. The feedgas flows between an outer, grounded, cylindrical electrode and an inner, coaxial electrode powered at 13.56 MHz radio frequency. The electric fields produced between the electrodes causes the gas to breakdown into a plasma state. While passing through the plasma, the feedgas becomes excited, dissociated or ionized by energetic electron impact. Once the gas exits the discharge volume, ions and electrons are rapidly lost by recomb-

nation leaving metastable species (e.g. O_2^* , He^*) and radicals (e.g. O). These reactive species are then directed onto a contaminated surface at high velocity where they can selectively neutralize CBW agents without damaging the underlying surface. Although the effluent of the APPJ may look somewhat like the flame of a Bunsen burner, its temperature can be maintained cooler than that of a hair dryer's exhaust. The reactive species of oxygen essentially "burn" many organic materials, such as CBW agents, at these relatively low temperatures (e.g. $\sim 70^\circ C$) without actually damaging the underlying material.

The reactive effluent of the APPJ has been shown to kill *Bacillus globigii* (BG) spores, a surrogate for Anthrax, with a D value (time to reduce viability by a factor of 10) of 4.5 sec at an exposure temperature of $175^\circ C$ and a stand-off distance of 0.5 cm. This is 10 times faster than hot gas at the same temperature and requires $\sim 80\%$ less energy input to achieve the same level of kill. Through active cooling of electrodes, we have also demonstrated 2 log reduction of BG spore viability within 30 sec at an exposure temperature of just $70^\circ C$. At this temperature there is essentially no pure thermal kill of bacterial spores and no thermal degradation of most materials.

Research efforts during FY01 have been directed toward's reducing helium consumption and increasing the allowable standoff distance. As a first step, we have developed a Plasma Decon Chamber for sensitive equipment and materials that can be placed inside a chamber. By recirculating the feed gas through a closed-loop system we are able to greatly conserve on helium, as well as alleviating any concern over reaerosolization of agent. A closed system also allows employment of methods such as pressure reduction and/or pulsing to greatly increase penetration of reactive species into contaminated equipment. The combination of heat, vacuum, forced convection and reactivity should enhance the removal of liquid agents off of surfaces, and what isn't adequately neutralized within the chamber will certainly be destroyed as it passes through the discharge during recirculation. This has been clearly demonstrated in our lab on simulants. Operation at $70^\circ C$ with high gas flow is more than adequate to evaporate the more volatile simulants, such as phenyl half mustard, causing them to be thoroughly decomposed as they make multiple passes directly through the plasma during recirculation, resulting in decon times of under 2 minutes. The less volatile simulants, such as Malathion which is 50 times less volatile than VX at $70^\circ C$, have to be decomposed on the contaminated surface. We have reduced the operating pressure from atmospheric (600 torr at Los Alamos) to 30 torr to increase the effective range of reactive species, allowing us to decon Malathion contaminated surfaces at 10 cm in under 5 min. Preliminary experiments being conducted at the Army's Dugway Proving Ground on actual VX appear to be consistent with simulant results.

The Uniform, Steady-State, Atmospheric DC Plasma

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For sterilization, surface modification, and other applications discussed at ElectroMed2001, atmospheric pressure plasmas are most convenient. Most such plasmas require radio frequency fed dielectric barrier discharges. Here we discuss a direct-current fed atmospheric pressure discharge (patent allowed). One great advantage of such a discharge is that it can also be produced by 60 Hz AC power from an inexpensive neon sign transformer. We have produced several liters of such plasma in atmospheric pressure helium, with smaller volumes in nitrogen and air. The maximum power applied is 300 W, limited by the present power supply. The longest steady-state runs are about 30 minutes long. Preliminary estimates of the ion density are 10^{10} exp 11^{10} per cc in helium.

The method of producing this plasma is using electrodes composed of unglazed ceramic moistened with water. Other resistive electrodes using carbon also work for a time, but the discharge shortly contracts into an arc, permanently damaging the electrode. Metal electrodes immediately arc.

One question concerns the uniformity of the helium discharge. Langmuir probes are difficult to use due to the high pressure and the high DC electric field. We have resolved this question using a combination of a photomultiplier tube and a current probe on the cathode circuit. We found to our surprise that the discharge is pulsing at 10 kHz. We use this feature for our diagnostics. The light pulses occur in phase with the current pulses. Scanning the photomultiplier over the plasma volume, we find that there seems to be no gross variation in the light output at different locations in the plasma volume. Also, the pulse light output seems to be rather constant from pulse to pulse. Hence we conclude that the discharge is rather uniform over the volume, rather than spatially fluctuating over time.

We are developing novel diagnostic methods for density measurements that are applicable to all steady-state atmospheric plasmas. One technique

is the double drift probe, reported on at ElectroMed1999. Other methods are undergoing testing. We have also found that the structure of the plasma sheath in a high-pressure gas is different from that in a free-fall Langmuir sheath, and have developed an analytical description for it.

Biochemical Pathways in the Interaction of Non-Equilibrium Plasmas with Bacteria

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To date, most research on the interaction of non-equilibrium, atmospheric pressure plasma discharges with bacteria has concentrated on the germicidal effects [1]-[4]. Therefore, published results deal mainly with killing efficacy and little attention is given to the biochemical pathways and their potential alterations when cells of micro-organisms are exposed to the plasma.

In this paper, an attempt to investigate the effects of plasma exposure on the biochemical pathways of bacteria is presented. In order to carry out such a study, a sub-lethal exposure to plasma is administered. The affected cells are then inoculated into various carbon substrates to evaluate any changes in substrate utilization relative to control cells. These changes are presumed indicative of plasma-induced changes in enzyme activity.

In addition, we present plasma killing experiments and using a scanning electron microscope, we investigate if any gross morphological changes take place when cells are exposed to a lethal dose of plasma. We are testing the hypothesis that disruption of the cell membrane, sometimes to the point of cell lysis, is the mechanism whereby plasma kills cells.

The discharge used in this study is generated, at atmospheric pressure, between two planar electrodes. The working gas consisted either of helium or a mixture of 97% helium and 3% oxygen. The plasma, which is weakly ionized and non-equilibrium, is allowed to come in direct contact with the samples under treatment.

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Production of NO for Medical Applications Using Pulsed Power Technology

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Recently, nitric monoxide (NO) has been widely used in medical treatments of acute respiratory distress syndrome (ARDS), high blood pressure and failure of breathing as it is an endothelium derived relaxing factor. A gas cylinder of N_2 mixed with a high concentration of NO is currently used for medical treatment in hospitals. This arrangement is potentially risky due to the possibility of an accidental leak of NO from the cylinder. Therefore on site generation of NO would be very desirable for patients with ARDS and other related illnesses.

We have recently reported on production of NO using a pulsed arc discharge [1, 2]. In the present work the discharge reactor is simplified and smaller than the latter. NO is generated by pulsed arc discharge in dry air. A maximum concentration of NO was 450 ppm with NO_2 of 150 ppm was achieved. NO_2 , O_3 and particles of brass electrodes, which are formed by the arc discharge, must be extracted from the gas before clinical inhalation. NO_2 produced in the discharge was successfully changed to NO using heated molybdenum. The concentration of O_3 was zero as determined by UV absorption measurements. The density of the brass particles, which have diameters of over 0.5 μm , was less than 1.39 mg/l. A filter could readily capture the brass particles. The final compositions of the gas mixture was 550 ppm of NO, 50 ppm of NO_2 and the balance N_2 at 1 atm.

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An Improved Plasma Reactor Configuration for Surface Treatment And Sterilization Based on the One Atmosphere Uniform Glow Discharge Plasma

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We have developed a glow discharge fourth-state-of-matter plasma, the One Atmosphere Uniform Glow Discharge Plasma (OAUGDP), that can operate at one atmosphere in air [1,2], and the active species from which are capable of producing a variety of plasma processing effects, including sterilization in a few tens of seconds at room temperature [3]. At the 1st ElectroMed Conference, we reported the rapid sterilization of a variety of micro-organisms by direct exposure to a volumetric OAUGDP generated between parallel plate electrodes [3], and we also reported sterilization by remote exposure to active species convected by an airflow from flat panels covered by a surface layer of OAUGDP to a remote chamber[4]. We demonstrated that the lifetime of the active species responsible for sterilization was sufficiently long that recirculating the gas flow decreased the killing time relative to that observed with a single pass of the airflow past the sample [3,4].

We have combined the best features of both previous designs into a new configuration that incorporates a closed-loop, recirculating airflow with a combined parallel plate and remote exposure design [3,4]. The new configuration incorporates recirculating airflow in a closed loop, which makes possible the build-up of high concentrations of sterilizing active species, while confining these species within the reactor where they pose no hazard to surrounding personnel. The new configuration also takes advantage of volumetric production of active species between parallel plates, while convecting these active species to an adjacent remote expo-

sure chamber where samples and workpieces can be treated without direct exposure to the plasma between the parallel plates. Data will be reported on the power efficiency, and the relative amount of the RF energizing power that appears in the plasma, and in reactive power that does not contribute to the formation of active species.

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Plasma Treatment of Biological Materials for BioDetection

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The unambiguous detection and characterization of biological warfare (BW) agents in a timely manner represents significant challenges. Unfortunately, the materials and technology to field weapons of mass destruction using biological warfare agents are increasingly available to rogue groups and nations. In order to protect troops and be able to fulfill mission requirements, commanders must have access to accurate information about biological threats. This requires near-real time detection of threats. A Matrix Assisted Laser Desorption Ionization Mass Spectrometer (MALDI-MS) is being developed to fill this need (Johns Hopkins University Applied Physics Lab). The MALDI-MS obtains spectra of the sample and based on peak identification, the sample is identified. *Bacillus anthracis* is one potential threat agent that is spore-forming. Spores have been shown to produce relatively few peaks without pretreatment prior to MALDI-MS analysis. Potential pretreatments may involve heat, chemicals, or electric fields. Because the device is intended for fielding, it is desired to minimize both consumables and power requirements. Therefore, we investigated the possibility of using a plasma discharge as a pretreatment method for the MALDI-MS process. This requires no consumable liquids and requires less power than heat treatment. The experimental data compared untreated samples of spores, vegetative cells and proteins with treated samples to determine if MALDI-MS spectra were improved with the use of treatment. Battelle Pacific Northwest National Laboratory collaborated with us on this effort, providing all MALDI-MS results. Results for spores, vegetative cells and proteins will be discussed. Defense Advanced Research Projects Agency Special Projects Office funded the work.

Reduction of *Bacillus Subtilis* and *Aspergillus Niger* Spores Using Non-thermal Atmospheric Gas Discharges

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Nonthermal gas discharges at atmospheric pressure such as dielectric barrier discharges are currently investigated for packaging sterilization in order to reach conditions required for aseptic food packaging. Especially understanding the basic reduction mechanisms and the intensification of the main reduction pathways are the goals of our investigations. For this we carried out germ reduction experiments with *bacillus subtilis* and *aspergillus niger* spores under different gas atmospheres and plasma conditions with dielectric barrier discharges. In order to analyze the contribution of the UV-radiation during plasma germ deactivation, experiments with different excimer UV-lamps driven also with dielectric barrier discharges in special UV emitting gases had been carried out.

Results of germ reduction experiments in barrier discharges and outlooks of atmospheric discharge arrangements using alternative electrode geometries suitable for packaging sterilization will be presented.

Microwave and Millimeter Wave Abstracts

Magnetic Field-Induced Stress Response in Biological Cells by Use of Cell Phones

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We have shown that electromagnetic (EM) fields induce the cellular stress response in the absence of elevated temperature, and therefore could be the primary biological effect in cell phone use. Low frequency magnetic fields are among the wide range of environmental stimuli that induce the human stress response gene HSP70, the normal physiological response to potentially harmful stimuli. Induction of the stress response by magnetic fields occurs at 14 orders of magnitude lower energy density than with thermal stimuli, the current benchmark for cell phone safety standards (1-4). On the basis of our observations, the EM-field-induced stress response is a more reliable and realistic criterion for cell phone safety standards. The significance of EM field-induced stress vis-a-vis thermal stress is underscored by a number of clear biological differences. Unlike the thermal stress response:

- magnetic field stimulation *does not* inhibit normal basal cellular protein synthesis (4);
- the EM field stress response is mediated through: specific DNA sequences (5).
- EM fields use the MAP-Kinase signal transduction pathway and induce binding-activity of pathway-specific transcription factor AP-1 (4, 6).
- the EM field stress response can be restimulated with higher or lower field strengths (4).
- the fact that the EM field stress response is induced at much lower energy densities may be the basis for all of the above differences.

Methods:

HeLa cells, transfections (1-3), protein lysates (1-4), HSP70 promoter constructs (2,3), c-myc protein expression vector (2,3), DNA sequence of plasmids by the DNA Facility of the Cancer Center Columbia University,

site-directed mutagenesis (Quikchange Stratagene), band shift analysis used mutated oligonucleotides as competitors (1-3) to ascertain sequence specificity, magnetic field exposure of transfectants, CAT and luciferase assays (1-5). Results were quantified using PhosphorImager and ImageQuant software. Electromagnetic field exposure conditions, Helmholtz coils, mu metal shielding and heat shock (1-6).

Conclusion:

The stress response induced by EM fields can be clearly differentiated from that induced by heat shock, because the two stressors affect different biological markers. Since stress proteins are induced at markedly lower energy levels by EM fields, they can serve as biomarkers to monitor cellular reactions well before the effects of increased temperature. Cell phone safety standards based on thermal effects are unrealistic, and should be replaced by standards that are biologically based.

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A Mechanistic Consideration of Localized Sub-Molecular RF Energy Absorption and Consequent Phonon Dispersion

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It is the overwhelming consensus of the bioeffects research community that radiofrequency (RF) energy may be absorbed by and interact within biologically viable media. The research basis on which this understanding is founded extends to the 1940s at which time RF energy radiating products were introduced for military and commercial applications. Subsequently, investigations into the biological effects of RF energy have continually evolved from evaluation of whole-body to non-uniform partial-body, and localized exposures. This has become a particularly important field of research in view of the recent demand for RF energy emitting devices operated in the near-zone of radiation sources. The progression from whole-body to localized exposure research has been necessitated by the widespread use of personal products providing such exposures. Notwithstanding the broad research activities to this time, we find that there remains an opportunity for a more refined level of biophysical experiment and analysis which embodies consideration of energy flow and activity commencing at the point of conversion - that is, the point of absorption. It is becoming increasingly apparent that a continued focus of investigation at the macroscopic level fails to fully incorporate electromagnetic and thermodynamic processes at the molecular, sub-molecular, and atomic levels. The oft-posed arguments distinguishing between ionizing and non-ionizing radiation fall short of fulfillment by virtue of well-intentioned misinterpretations of fundamental principles of solid-state physics. Such arguments give inadequate consideration to covalent bond dissociation energy states which may be realized due to multiple absorption events - a physical mechanism not available to the single-event ionization process. A paradigm shift to adopt metrics related to molecular, sub-molecular, and atomic level energy absorption and conversion may eliminate the seemingly insurmountable task of continuing exclusively with present research methodologies to determine mechanisms of RF energy interaction and any consequent results. Obser-

vation techniques not available at the outset of the bioeffects research endeavor, commenced some fifty years ago, may be employed today to characterize energy absorption parameters at the molecular, sub-molecular, and quantum mechanical level and productively shift consideration away from an artificially imposed limit of averaging related to mass and volume. In so doing determination of energy fluence at the point of action may be modeled initially and experimentally verified subsequently. RF energy absorption by and transport within lossy media such as, for one example, human deoxyribonucleic acid (DNA) is generally non-uniform and gives rise to localized sub-molecular energy absorption hot spots that escape observation and characterization by measurement methods employed to date. Localized segments of macromolecules exposed by virtue of a preferred molecular configuration, such as the supercoiled DNA molecule, and the non-isotropic configuration of RF energy sources are anticipated to provide the environment for non-uniform phonon dispersion resulting from preferentially localized photon incidence. The phonon absorption spectrum and consequent phonon dispersion within macromolecules, such as the human DNA molecule, have not been fully investigated or characterized and offer the prospect for a more thorough understanding of the interaction mechanisms giving rise to many of the, seemingly, unexplainable effects reported to date. The challenge of expanding biophysical research activity beyond the thermal/ionizing/non-ionizing conundrum to investigation of: localized photon density; translational, rotational, and vibrational modes; and the phonon energy density of states (a virtual continuum for macromolecules such as human DNA) provides the opportunity for a more complete understanding of RF energy interactions in concert with the full ongoing research effort related to this field.

**Radiofrequency Energy Absorption
Characteristics in a Heterogeneous Model
as a Function of Complex Wave Impedance
Variations Proximal to a Current Source**

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In the near-zone of a current element wave impedance is not a constant of the medium but exhibits a dependency related to distance to the current source generating electric and magnetic fields of interest. When considering heterogeneous tissue models in conjunction with full field representations it is shown that prediction of specific absorption rate, based on internal field intensity, may be significantly underestimated by simplifications that may be employed for computational convenience or experimental efficiency. The full field representation/impedance technique employed for multi-laminate media, such as a six-layer human head model, indicates that specific absorption rate is functionally dependent on each individual layer thickness of a multi-laminate structure comprised of lossy media. In view of the spatial dependency of wave impedance, and consequently energy absorption, an impedance matching effect, or near matching effect, may encourage reconsideration of some methods now employed to determine energy absorption rate. In particular, the significant variability of bone and subcutaneous fat layer thickness corresponding to humans of different age and physical stature indicates that energy absorption rate documentation will be more accurate and complete by employing a plurality of structures that embrace that variability. The accompanying illustrations confirm the effects for a number of heterogeneous profiles.

Nocturnal Melatonin Excretion and Work on VDU

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Introduction:

Electromagnetic fields emitted by Video Display Unit (VDU) have been shown to induce a severe decrease of melatonin in young chickens. [1] The only study conducted on VDU workers showed that circulating melatonin decreased significantly during the days of VDU work, compared to days of leisure. [2] Some studies have reported decrease of urinary excretion of 6-hydroxymelatonin sulfate in railway and electric utility workers, exposed to EMF of Extremely Low Frequency. [3] [4] [5] Because of the fundamental role of melatonin in chrono-biological and hormonal regulations, and considering that VDU are also ELF emitters, it seems of great interest to evaluate the influence of VDU work on nocturnal melatonin excretion.

Materials and Methods:

Women, aged 20 to 40 from Burgundy region were selected for the study. Participation required that subjects take a contraceptive pill, monophasic minidosed, have no psychotropic treatment, no psychiatric disease, nor have had jet lag more than 1 hour during the last 2 months. Exposed group: -6 persons, secretaries, working on a VDU more than 4 hours /day, 5 days/week, for more than a month. Control group: -7 persons, who didn't work on a VDU, watching TV less than 2 hours/day and at least at a distance of 3 meters. The nightly urine sample was collected at the end of a week of work, from the night of Friday to Saturday (20 PM to 8AM). The urinary concentrations of 6 sulfatoxymelatonin (6-OHMS) were determined by the Radioanalysis and Radiopharmacy Service of the Neurocardiological Hospital of Lyon.

Results:

The collected data show a very significant decrease of nocturnal 6-

OHMS urinary excretion in the group working on VDU's compared to controls. The average decrease was of 54.08% for the exposed group compared to the controls, with $p < 0.005$ (Mann-Whitney test). These data suggest that work on VDU can affect melatonin secretion and further research is needed on a larger cross-section of population, including measurements of the fields which each individual is exposed to.

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Non-Thermal Effects in Hyperthermia

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Objectives:

Hyperthermia is a rapidly developing treatment method in oncology. The classical effect is based on well-focused energy absorption targeting the malignant tissue, for which local temperature had been the main characteristic parameter. However, the efficacy of treatment also depends on non-thermal factors: stress protein (SP) synthesis, and non-linear thermodynamic effects (e.g. modified pressure and electric current gradients). These factors are responsible for rendering most classical hyperthermia treatments ineffective. This review discusses these non-thermal phenomena and their use in increasing the efficacy of hyperthermia.

Stress Protein Synthesis:

Cells produce SPs in response to heat [1], magnetic fields [2], and other physical stresses [3]. Intracellular SPs allow the cells to adapt to stress and prevent significant damage to them by stabilizing essential proteins and suppressing apoptosis. Tumors can therefore develop resistance against heat, and significantly reduce the effectiveness of classical hyperthermia. Furthermore, SPs can reduce the chemo- and radio-sensitivity of the cells; thus eliciting a negative effect [4]. SPs secreted in the cellular membrane and in the extracellular matrix (ECM), however, enhance apoptosis [5], support tumor-specific antigens [6], and stimulate lymphoid cells [7]. While classical hyperthermia treatments heat entire regions, including the cytoplasm of the cells within the targeted tissue, a new method, electrohyperthermia, has been developed to target the ECM of the malignant tissue by using a capacitively coupled electric field. Transmitted energy is absorbed in the ECM, not in the cytoplasm within the chosen frequency range and field-strength.

The cytoplasm is only heated by slow heat diffusion, which delays intracellular SPs synthesis until the damage becomes irreversible.

Thermodynamic Effects:

Heating of the ECM increases ion-mobility and intensifies the metabolic rate. The temperature gradient on the membrane, guided by the Onsager-relation, also alters cell membrane permeability, generating extra ionic influx, which increases membrane potential and osmotic pressure within the cell. All of these effects promote cell membrane damage and lead to cell death.

Discussion:

Based on this theory, several electro-hyperthermia devices have been developed to target a wide range of malignant sites with great success: a DC system for surface treatments, an AC device for intracavitary treatments, and an RF-capacitive coupling system for deep-seated tumor treatments. The inhomogeneity of the complex dielectric constant and vascular properties of malignant tissues also further aid in focusing the energy to malignant areas with these devices. The clinical results show a significantly improved coupling with other treatments (e.g. chemotherapy, radiotherapy, and surgery) and a drastic improvement over classical hyperthermia.

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Stress-Limiting Effect of Low Intensity Microwaves

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Objectives:

At the present time, due to its therapeutic efficacy, the low-intensity millimeter electromagnetic radiation (EMR) is gaining wider use in the practice of medicine. To analyze the mechanisms of this effect of EMR, we studied how the millimeter waves contribute to the development of a stress reaction, which accompanies any ailment as a non-specific component.

Methods:

We studied 5.6 mm, 10 milliwatt/cm² electromagnetic waves created by the "Yav' 5.6" generator. All experiments were carried out on white rats of the same weight, sex, age and individual characteristics as determined by the "open field" test. Stress was created by placing the animals in hypokinetic conditions, that is by housing the animals in custom-made "pencil box"-type plastic enclosures. All animals were separated in 4 groups, 8-10 animals per group. Animals of the 1st group were housed in regular vivarium conditions (biological control). Animals of the 2nd group were also housed in regular condition, but we exposed to EMR daily. Each exposure session lasted for 30 minutes per day, for 9 days, with radiation focused on the occipital area. The 3rd group was comprised of animals kept for 9 days in hypokinetic conditions in "pencil box" enclosures. The 4th group was comprised of animals subjected to both hypokinesia and EMR as described above. Animals from groups 2 and 4 were exposed to EMR simultaneously. We measured non-specific resistance to stress based upon cytochemical parameters of neutrophils (levels of peroxidases, cation proteins and acid phosphatase), parameters of lymphocytes (levels of succinate glycerophosphate dehydrogenase), state of peroxide oxidation of the lipids and thiol-disulfide exchange in various areas of the central nervous system.

Results:

All hypokinetic animals developed a stress reaction characterized by

a precipitous decrease of all parameters of non-specific resistance, activation of the lipid peroxide oxidation processes and thiol-disulfide exchange. Their changes peaked by the 9th day of movement limitation. Animals with no movement limitations but exposed to EMR showed a 10-15% increase in the neutrophil cytochemistry, as well as an increase in the -active products in thalamus and hypothalamus against the background of decreasing total thiol groups. Neither the latter phenomenon, nor a decrease in the non-specific resistance, based upon brain metabolism, were shown in the hypokinetic animals exposed to EMR. Moreover, the levels of lymphocytic dehydrogenase activity in these animals was higher than that of the control animals.

Conclusion:

We postulate that development of the stress reaction in the hypokinetic animals is alleviated by the effect of EMR. This coincides with our clinical observations. Perhaps, the ability of electromagnetic waves to prevent stress could mediate its therapeutic activity.

This work was supported by the Tavrida National University.

A Study on Biological Effects of Millimeter Waves

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The existence of nonthermal effects, particularly resonant effects, of low-intensity millimeter waves has been a matter of controversy for nearly 30 years. There is a large number of publications where nonthermal and resonant effects on cellular and subcellular structures are reported, discussed and used in therapeutical applications [1]. But there exists an equally convincing body of studies, which indicates that the effect of millimeter waves is nonresonant [2]. Experimental studies on the biological effects of millimeter waves have been performed over a wide range of frequencies; however, certain frequency ranges have been preferred over others, possibly due to the availability of millimeter wave systems at these frequencies. One such frequency range is the 41 GHz to 43 GHz range, where resonance effects on the cell growth rate [3], as well as strongly nonlinear, resonant effects on DNA [4] have been reported. We have focussed our research on this frequency range and have studied the response of cells and DNA to these electromagnetic waves, using four different, but complementary diagnostic techniques. The millimeter wave generator provided a CW output up to 1.5 W (adjustable), corresponding to a maximum intensity at the sample of 68 mW/cm². Since previous studies in this frequency range have used *E. coli* as model [4,5], we have, following these studies, focussed first on the growth rate of *E. coli* and secondly on the absorption spectrum of *E. coli* in the range from 41 GHz to 43 GHz. From studies in other frequency ranges [5], it is known that resonances in the absorption spectrum correspond to subcellular resonances, e.g. in DNA. We have consequently, on the subcellular level, studied the effect of millimeter wave radiation on plasmid DNA, α *Luc*, purified from *E. coli*, using electrophoresis and bacterial transformation. All of the studies provided negative results with respect to the existence of resonance ef-

fects. Initially observed resonance-like features in the absorption spectrum of *E. coli* turned out to be an artifact caused by frequency dependent reflections in the millimeter wave system. Such internal reflection might explain the "resonant" effects reported in the literature.

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Plasma Abstracts

Comparative Analysis of Various Types of Electric Discharge for Disinfecting of Sewage

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One of the ways to improve environmental safety is to find new methods and technologies of effective antimicrobial cleaning of both domestic and manufacturing sewage.

It is well known that electric discharge in liquid is an effective destructive agent for various germs. Investigations of the effect of an electric discharge in a liquid on a microbe consist of two major components: 1) exploration of the mechanism of such effect and, 2) development of specific facilities to use this effect in a commercial sewage cleaning.

Major requirements in sewage treatment facilities design, especially those utilizing a sewage flow, are high efficiency of decontamination and the facility's durability which depend on the discharge type and the electrode system used to form the discharge.

This work presents a comparative analysis of decontamination efficiency of two discharge types: arc discharge and plasma breakdown in electrolytes. These discharges were formed by various electrode systems, which may be considered prototypes of commercial devices.

As a treated medium the sewage from one Moscow suburbs -with both separated solid phase and unseparated solid phase- was used. The main biological pollutants of the sewage included: *E.coli* and other enterobacteria, saprophytic microflora, helminthes-eggs, Metazoa invertebrates etc. Bacteriological tests were performed according to the method used in [1] both immediately after discharge effect and 12 hours after treatment. *E.coli* test parameter, residual chlorine and pH change were determined. The experiments were performed in a motionless medium and in a flow.

It was found that the arc discharge demonstrated the best characteristics. Minimum energy input needed for complete microbe destruction was about 3 KJ/L. Residual chlorine evolving in the arc discharge took place under less energy input than that of the plasma breakdown in electrolytes. A slight increase of pH (10 to 15 %) was observed. The increase was not dependent on the discharge type and total energy input.

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Control of VOCs and PM_x in Indoor Environments by Air Ionization

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The generation of non-thermal plasmas containing reactive oxygen species (ROS) at ambient temperatures is a rapidly developing area of technology. Recent developments in the application of controllable air ionization processes in indoor air environments has led to significant reductions of specific volatile organic compounds (VOCs) and fine particulate matter (PM_x) with minimal formation of byproducts. Removals of airborne microbes and neutralization of odors are also enhanced by air ionization. It is postulated that the process of air ionization involves the electronically induced formation of small air ions, including superoxide, the diatomic oxygen radical anion, O₂⁻, which react rapidly to oxidize VOCs and neutralize PM_x. The significance of air ionization chemistry and its potential for contributing to significant improvements in Indoor Air Quality will be discussed.

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Utilization of a Glow Discharge Plasma at Atmospheric Pressure for Sterilization of Surfaces

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Glow discharge plasmas have been used in a number of ways including the etching of semiconductor surfaces, the modification of the surface characteristics of textile fibers, and the coating of metal surfaces. In many cases, the need for complex vacuum systems hampers the industrial development of plasma devices. At North Carolina State University, a collaborative effort between the Departments of Nuclear Engineering and Food Science is exploring the utilization of atmospheric pressure glow discharge plasmas for the sterilization of surfaces. The experiments are performed in PALADIN a parallel plate glow discharge device powered with 250 watts of radio frequency power. PALADIN does not use a vacuum system, however various mixtures of air and other gases can be used. Samples of bacteria (*Lactococcus lactis*) and bacteria-phage have been exposed for various times and powers in PALADIN. In addition to testing prepared samples of bacteria and bacteria-phage, lettuce samples have been exposed. All samples have shown several log reductions in bacteria or bacteria-phage populations as a function of time of exposure. This paper reports the experimental methods and recent results and discusses the theory and sterilization mechanisms of this technique.

Investigation and Development of Ozonators for Medical Purposes

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Ozonotherapy may become socially-important medicine direction. Simplicity of application, high medical and economical efficiency are characteristic features of this treatment. Atherosclerosis, surgical skin and venereological pathologies, infection diseases, etc. could be treated with ozonotherapy.

Nowadays a number of ozonotherapeutic treatment technologies, using both low (Russian school of ozonotherapists) and relatively high (foreign school) ozone concentrations, are well developed. Utilization of these technologies require special equipment - medical ozonators with wide range of output O_3 concentrations and velocities of output fluxes and high syntheses precision in all range.

The results of investigations and developments performed in RFNC-VNIIEF in the field of barrier discharge, high-voltage high-current electronics and automation, oriented on creation of ozonators for medical purposes are presented in this paper.

Development of the electrode systems and discharge chambers, power sources resulting from barrier discharge research leading to stable ozone accumulation with precision not less than 10% in a wide concentration range are presented.

Several ozonators were fabricated based on the results of our investigation. An ozonotherapeutic apparatus with an output concentration range

of 0.1-80 mg/L was produced. The apparatus provides 4 automatically adjusted velocities of ozone-oxygen mixture output: 0.1, 0.25, 0.5 and 1 L/min. It also provides fast filling of external treatment chambers. Power consumption is 30 W. Parameters of the apparatus make it possible to provide many treatment regimes for both Russian and foreign schools of ozonotherapists.

Medical support of the development was provided by Russian Ozonotherapists Association and Federal Agency of Biomedical and Extreme Problems.

Russian Ministry of Health recommended the ozonator for production and application in medical practice.

Study of High-Voltage Diffused Gas Discharge Effect on Microbiological Cultures

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The results of our investigation involve the inactivation of microbiological cultures in plasma of diffused low-power pulsed-periodic discharge (average current is 1.10 mA), formed in air gaps with interelectrode gap of 10.15 cm, are presented. The goal of the investigation is the development of gas-discharge technology for medical disinfection (including porous and heat-sensitive materials) of boxes of large volume.

Plasma Sterilization in Radiofrequency Discharge

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This paper clarifies the conditions under which the process of plasma sterilization of medical tools may be efficiently performed in RF capacitive gas discharge of low pressure in air. Experiments were performed with a number of gram-positive and gram-negative bacteria as well as with fungi. The process of sterilization in the RF discharge is shown to possess a threshold pattern. It is theorized that the bombardment of bacteria with positive ions and hot molecules of the neutral gas is the main sterilizing factor in low pressure RF discharge, and that UV radiation from the plasma plays the auxiliary role.

Experiments on plasma sterilization were performed in a discharge chamber 100 mm in diameter and an interelectrode gap of 54 mm. A RF capacitive gas discharge in air was ignited in the chamber within a pressure range $p = 0.1$ -1 Torr. The RF voltage and the 13.56 MHz operation frequency was supplied from a generator connected to one of the electrodes. The other electrode was grounded. Gauze with 0.5-cm mesh size was installed between the electrodes completely occupying the cross-section of the discharge tube. Samples under study with bacterial cultures were put on this gauze (needles were used as sample holders). The following cultures were put on the needles: gram-positive (*S. aureus*, *S. epidermidis*, *Str. mitis*) and gram-negative (*K. pneumoniae*, *P. mirabilis*, *E. cloacae*, *E. coli*) bacteria, as well as fungi (*Candida*). The following main factors may affect the micro-organisms in the RF discharge in air at low pressures: i) flows of charged particles (electrons, ions); ii) heated neutral gas; iii) UV radiation; iv) low pressure (vacuum). Our studies show that storing samples in the vacuum chamber with the inner air pressure from 0.1 - 10 Torr over a period of 12 hours does not decrease the quantity of microbes in the sample. Therefore the vacuum chamber has no noticeable effect on bacterial cultures stored within it. Some culture samples were processed with only the discharge radiation by putting them outside the chamber but close to the quartz wall

in the region of maximum radiation at $W = 400$ W for 5 min. This processing technique did not affect the initial number of bacteria on the sample. For comparison under the same conditions the samples inside the chamber were sterilised within 5 s after switching on the discharge. Therefore the discharge UV radiation by itself does not kill bacteria, and some other factors are needed for sample sterilization. The gauze with the samples on it was under floating potential and the decelerating potential of 10-15 V stopped the bulk of electrons, and only a small portion of the fastest electrons could reach the samples. Consequently, the contribution of the electron flow to the sterilization process was not substantial. The voltage drop accelerated ions. The accelerated ions bombarded the surface of bacteria demolishing their protective outer shells. If one measures the flux of positive ions on the sample and multiplies the threshold time t_s , at which one observes the step-like decrease of the number of bacteria on the sample by the ion current value I_i , then the product $D = t_s I_i$ remains approximately constant. It is theorized that for a bacterium to be killed a certain dose of ion irradiation must be delivered, i.e., its shell should experience a certain amount of collisions with accelerated ions. Hot gas molecules may cause heat damage to the outer shells of bacteria causing their death. At an air pressure $p = 0.6$ Torr even at the lowest RF voltage, $U_{rf} = 210$ V, the neutral gas temperature is approximately 100°C , and at $U_{rf} = 600$ V the gas temperature is 200°C . The processing occurs at the lowered gas pressure, and for heating bacteria one requires more time than in air at atmospheric pressure. The higher the temperature of the neutral gas, the less time required for heating bacteria to death temperatures. Consequently, bombardment of bacteria with the flows of positive ions and hot molecules of neutral gas are the main sterilizing factor in low-pressure RF discharge. We suspect that UV radiation from plasma plays an auxiliary role.

Installation on Plasmachemical Disinfection of Hazardous Medical Waste

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The technology of plasma-chemical disinfection of hazardous medical waste by method of high-temperature mineralization, with the application of low-temperature plasma produced by a plasma generator, is described. The method allows not neutralization of medical waste, but also reduces the volume of waste being buried by 50-400 times. The organization of the waste combustion process is as follows:

Two-stage combustion in the furnace at temperature of 1000-1200°C and in the afterburning chamber at temperature of 1200-1300°C with a dwell time of flue gases no less than 2 s; Obligatory quenching (fast cooling) of flue gases; Multistage cleaning of flue gases of fly ash, vapors of heavy metals, acid gases, and, if necessary, dioxins and furans; Automated control of mode parameters, ejection into the atmosphere and control of all technological process; Low consumption factors on energy and reagents.

The installation is designed for treatment of 150 ton/h of hazardous medical waste. The rotary kiln with parallel-current flow of burned waste and incandescent gases is used for plasma combustion of medical waste in the described installation. Parallel flow in a rotary kiln allows waste to constantly mix while burning, preventing fusion or baking in a layer and thus intensifies the processes of heat and mass exchange. The wastes are supplied to the furnace periodically through a system of trays with the help of pneumatic pushers. Two plasma generators are mounted on the loading end of the furnace, one on the afterburning chamber. Unloading of slag from the furnace is executed in the slag quenching device which is filled with water. After quenching and cooling the slag is sent for disposal. The gas cleaning system consists of a venturi scrubber, packed-bed scrubber, demister and adsorber.

The process allows us to solve the problem of complete destruction of hazardous medical waste by organizing, collecting and packing the waste, automation of the loading processes, organization of combustion regimes and a high degree of flue gases cleaning.

Enhanced Sterilization: A Comparison of Gases Utilizing an Atmospheric Plasma (OAUGDP)

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Atmospheric Glow Technologies was formed in May 2000 by researchers from the University of Tennessee. We have licensed the One Atmosphere Uniform Glow Discharge Plasma (OAUGDP) and are developing and commercializing its applications.

For many years, development of the OAUGDP has examined the role of air as the background gas from which the reactive oxygen species have been produced for the sterilization of micro-organisms. While the advantages of using room air are numerous, there are some applications that would benefit from the decrease in sterilization times gained by optimizing the oxidative gas chemistry.

We are reporting effects of using Air, Nitrogen, 50% mixture of Nitrogen and Oxygen, and pure Oxygen on both gram positive (*Staphylococcus aureus*) and gram negative (*E. coli*) micro-organisms. Data from additional experiments showing the inactivation of bacterial spores will also be presented. The destruction of the micro-organisms has been quantitated by viable counts and verified by epifluorescent microscopy utilizing a live/dead stain.

The Operating Conditions and Enhancements of an Atmospheric Plasma (OAUGDP) Parallel Plate Reactor

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Atmospheric Glow Technologies was formed in May 2000 by researchers from the University of Tennessee. We have licensed the One Atmosphere Uniform Glow Discharge Plasma (OAUGDP) and are developing and commercializing its applications.

In the fall of 2000, following the formation and setup of our new company, the standard design for the OAUGDP parallel plate reactor was re-examined and improved. The two key improvements in the parallel plate reactor's design were alterations to the cooling of the reactor electrodes and enhancements in the reactor's re-circulation pathway. By using air-cooled electrodes, the previous reactor's use of water-cooled electrodes has been shown not to be a critical requirement. This alteration increases the reactor's portability and simplifies the system.

The killing of micro-organisms in a parallel plate reactor originates from a variety of sources, the most common methods: ion bombardment, gas chemistry toxicity, and UV photons. In a Remote Exposure Reactor (RER/blower), the only means of killing is gas chemistry toxicity. This implies that a parallel plate reactor could be optimized via improved gas chemistry to enhance the initial killing times. This was accomplished by shortening the re-circulation path length on the standard parallel plate reactor design.

New research data generated using this reactor is detailed in our other presentations. Some of the operating conditions and reactor design for these other presentations will be presented. Additionally, data comparing

the previous reactor's killing curves to the new one will be presented, as well as our system's power consumption data.

Understanding Arthroscopic Ablation

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Approximately five years ago orthopedic surgeons were introduced to new radiofrequency electrosurgical tools, which were reasonably efficient in removing soft tissue while simultaneously coagulating blood vessels. The term ablation describing this process was introduced into the orthopedic vocabulary. These tools became integrated into various orthopedic procedures, and were used in nearly 500,000 surgical cases in 1999. In order to develop fundamental understanding of the physical principles of electrosurgery, an electrosurgical probe was studied both experimentally and theoretically under controlled conditions. The following issues were investigated: (a) Electrical characteristics of electrosurgical probes, (b) Spark formation and light emission by electrosurgical probes, and (c) Spark characteristics: temperature and brightness. It was found that the electrosurgical process proceeds in stages, as follows: 1) When energized, an electrosurgical probe initially dissipates radio frequency energy in the irrigation fluid, which is heated. 2) When a specific fluid volume is heated to boiling temperatures, steam bubbles are locally generated in the vicinity of the electrode edge. 3) The presence of a bubble intensifies the internal electric field strength, and decreases the threshold for a sequence of electrical discharges ("train of sparks") and plasma channel formation in the hot steam inside the bubble. 4) The plasma channel heated to high temperatures between 2,000 and 10,000 °C. The heated plasma emits intense flux of light (ultraviolet, visible and infrared). This light is radiated away from the plasma channel, which becomes visible as a spark. This intense light most likely plays an important role in tissue ablation (like intense laser light). 5) After a "dead time" during which no spark activity

exists, the next "train of sparks" is initiated in another bubble, and the process repeats itself as long as the probe is energized. Detailed results of the studies will be presented and discussed.

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Eradicating Biofilms with Atmospheric Plasma

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Atmospheric Glow Technologies was formed in May 2000 by researchers from the University of Tennessee. We have licensed the One Atmosphere Uniform Glow Discharge Plasma (OAUGDP) and are developing and commercializing its applications. Microbial biofilms are ubiquitous and can be found virtually anywhere there is moisture and a solid surface for attachment. Layers upon layers of organisms form intricate structures that are mechanically tenacious and resistant to chemical and antibiotic attack. According to CDC, over 3.5 million patients per year contract nosocomial infections at an estimated cost of \$4.5 billion. Consequently, the reduction to very low levels of micro-organisms on surfaces relevant to healthcare has become a significant area of effort.

We are reporting a novel method of biofilm destruction on medical and dental materials using the OAUGDP. Reactive oxygen species produced by the plasma attack and destroy biofilms. Biofilms of *Pseudomonas aeruginosa* were formed on glass and stainless steel coupons. Following direct plasma exposure of two minutes, a three log reduction was observed using viable counts. At four minutes, a seven log reduction resulted. Confocal epifluorescent microscopy utilizing a live/dead stain revealed cell death throughout the biofilm. We will report further studies with flow-cell constructed biofilms. Additional studies with Variable Pressure Scanning Electron Microscopy indicated there was no plasma-induced damage to important medical grade polymers and stainless steel following 4 minutes of exposure.

Pulsed Power Abstracts

Bacterial Decontamination of Water by Means of Pulsed Corona Discharges

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Whereas bacterial decontamination of gases by means of pulsed corona discharges is now a firmly established method, efforts to use pulsed corona or streamer discharges in liquids for decontamination have only begun recently [1,2]. This is due to the extremely high electric field intensities required to initiate plasma formation in liquids. Using a wire anode and a planar cathode, experimental studies have shown that electric field intensities of approximately 2.5 MV/cm at the wire surface are needed to obtain pulsed corona discharges in tap water. The electric field pulse needs to be short enough to prevent arcing, an effect which occurs when the first streamer reaches the opposite electrode. By using a tungsten wire with 75 μm diameter, two cm apart from a plane cathode, and applying a 600 ns, 135 kV square wave pulse, we were able to obtain a pulsed water corona discharge (PWC) without glow-to-arc transition. The effect of these discharges on bacteria was studied using water contaminated with *Escherichia coli* and *Bacillus subtilis*, the latter in both the vegetative and spore state. The strongest effect was obtained on *E. coli*. The concentration of *E. coli* could be reduced by two orders of magnitude after applying 3 corona discharges to the water. The corresponding energy expenditure is 4 J/ml. The decontamination rate had the largest values at the beginning, and decreased considerably after 15 electrical discharges, an effect which has also been observed in decontamination experiments using pulsed electric fields (PEF), and is assumed to be due to bacterial cell adhesion to and release from the chamber walls [3]. For *B. subtilis* in the vegetative state, it took 20 discharges to reach the same result, corresponding to an energy expenditure of 28 J/ml. There was no effect on *B. subtilis* spores. Comparisons with the PEF method [3,4], a more widely explored electro-technology used for bacterial decon-

tamination, indicate that the decontamination efficiency of the PWC method is slightly higher than that of the PEF method.

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Investigation into the Influence of Sewage Treated by Electric Discharge on River Ecology

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Cited literature data point out that antimicrobial properties of water treated by pulsed electric discharge are revealed maintained over a period of several months. At present a number of authors examine the possibility of using electric discharge for sewage decontamination, both domestic and municipal. The present engineering process is not changed as a whole but sewage chlorination is replaced by pulsed electric discharge treatment.

Cleaned and decontaminated sewage eventually merges with a river. Therefore there emerges a question pertinent to the influence of water treated by electric discharge river ecology.

This work presents the tentative results of the effect of sewage treated by pulsed electric discharge on the biological state of river water. As a treated medium the sewage from one of the Moscow suburbs - Podolsk city - was used. After sediment centrifugation the sewage was treated by pulsed electrical discharge and then diluted by water from the Pakhra river in ratio from 1:1 to 1:3. During the experiments different types of discharges (spark discharge, arc discharge and plasma breakdown in electrolytes) with various parameters were used. In all experiments the energy input exceeded the value necessary for full destruction of pathogens. According to the method used in [1] the *E.coli* index change was determined. The tests were performed immediately after mixing the sewage with river water, then 12 hours, 24 hours, 2 days and 7 days after mixing. Hydrobiological analysis of the benthos and the plankton was carried out in the same intervals.

Under the chosen discharge parameters and experimental conditions the period of reconstruction of the *E.coli* index of the river water to the

initial value was within 5 to 10 days after mixing with the treated sewage and is dependant on the dilution ratio. Hydrobio-assay of the benthos and the plankton revealed that at a dilution ratio of 1:1 the number of organisms and their activity are at a minimum even 5 days later. Under treated sewage - with a river water ratio of 1:3 during the same period there is an increase both in species diversity of saprobes and their physiological activity.

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Repair Time of Bacteria after Pulsed Electric Field Application

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Pulsed electric fields are widely used for bacterial decontamination of water and liquid food [1]. The decontamination mechanism is assumed to be field induced poration (electroporation) of the cell membrane. Depending on pulse amplitude and duration a certain percentage of the bacteria suffer irreversible damage, and consequently cell death. Those, which survive need a certain time to repair the field-induced damage. During the time it takes to re-establish the integrity of the outer membrane, the cell is more susceptible to the impact of second electric field pulse. By using two subsequent electric field pulses, and measuring the viability of the bacteria depending on the time between pulses, it is therefore possible to obtain information on the repair-time of the outer membrane, the time the cell "remembers" the previous pulse. The pulses were generated by means of a generator where IGBTs were used as closing and opening switches. The maximum voltage was 1.5 kV, at a maximum current of 160 A. The pulse duration of the square pulse (rise and fall time approximately 200 ns) was 4 μ s, the time interval between the pulses was varied from 4 μ s to 40 s. The load was a cuvette with plane 1 cm² aluminum electrodes, 1 mm apart and filled with a solution (LB Broth), which contained the *Escherichia coli* bacteria at a concentration of approximately $3 \cdot 10^5$ cells/ml. The viability of the *E. coli* after electric field application was measured by using manual counting for *E. coli* colonies in cultured agars. The results showed that independent of the field amplitude (in the relatively narrow range of 13 kV/cm to 15 kV/cm), the decontamination rate increased logarithmically from a value, which was obtained with two independent pulses to 1.5 times this value for pulses separated by a time corresponding to the individual pulse duration (4 μ s). The average repair time of the *E. coli* bacteria after electric shocks, defined as the time for which the difference in survival rate for

closely spaced and widely separated (60 s) pulses had increased to 50% of its maximum value is approximately 10 ms. This result is not only of scientific interest, but it shows that it is possible to increase the energy efficiency of bacterial decontamination using pulsed electric fields by more than 25 percent using pulsed power generators which operate at repetition rates exceeding 100 Hz.

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Comparison of Pulsed Electric Field and Heat-Induced Damage on *Escherichia coli*

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For a number of reasons, including potential spoilage and health related issues, the physiological properties of cells which survive inactivation treatments, such as Pulsed Electric Field (PEF) or thermal exposure, are important although these properties are not straightforward to assess. In this study we have compared the 'recovery time' of a PEF (30 KV/cm) treated *E. coli* population with a heat (55°) treated population. This was done using two different microbiological assay methods.

One of the methods employed was the standard plate count method which determined the number of *E. coli* cells which survived treatment based on the ability of the surviving cells (or cell clumps) to form colonies on agar medium (PCA) after 24 h incubation at 37°C. The second method involved automated conductance monitoring which gave a detection time (DT) based on the time taken for the surviving population to multiply and cause sufficient change in the conductivity of the growth medium (threshold value) to allow automated detection. This method was used to evaluate the specific growth kinetics (recovery time) of a similar number of *E. coli* cells which had survived either heat or PEF treatment.

The results showed that *E. coli* cells which survive PEF treatment require relatively little time to recover and multiply whereas cells which have survived heat damage exhibit considerable growth delay. This is consistent with there being much less severe residual damage in cells which survive PEF treatment and it may be suggested that PEF-induced killing (by electroporation) is essentially an all or none effect.

The finding that PEF does not produce large numbers of sublethally damaged cells which may subsequently recover and replicate has beneficial implications for food processing applications of PEF.

Pulsed Light Inactivation of *Escherichia coli* and *Listeria monocytogenes* in Liquids and on Test Surfaces

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There is considerable interest in the use of pulsed light for the disinfection and sterilization of liquids and materials. Research at Strathclyde University in this area has been mainly concerned with the development and application of high intensity, pulsed UV rich light for the destruction of spoilage and pathogenic micro-organisms. Experiments have been carried out to determine the microbiocidal effectiveness of pulsed light treatment when microbial populations are exposed in liquids and on different test and food surfaces such as fresh meat.

The light source employed was a low pressure (450 torr), Xenon filled flash lamp with a clear quartz envelope. The light source was driven from a stacked Blumlein cable generator which produced a pulsed duration of 280 ns and developed a peak electrical power output of 235 MW. The test bacteria were the foodborne pathogens *Escherichia coli* and *Listeria monocytogenes* and these were inoculated at known densities into liquids and onto test surfaces prior to exposure to pulsed light. Under the conditions used a negligible rise in temperature occurred at exposed surfaces so that no apparent photothermal effects were involved and so lethality could be attributed to the photochemical effects of the shorter UV wavelengths.

With both *E. coli* and *L. monocytogenes*, a 7 log reduction was apparently achieved when these organisms were exposed to pulsed light in clear liquid or on the surface of agar plates. Further experiments, however, demonstrated that a considerable fraction of the bacteria which appeared to have been inactivated (ie were unable to replicate and form colonies) on the agar surface could, in fact, regain the ability to replicate following

homogenization in liquid and seeding onto fresh media. Incorporation of this procedure yielded only a 3-4 log reduction when the test organisms were exposed on an agar surface or only a 1-2 log reduction when a meat surface was used. Experiments are ongoing to investigate the reasons for these effects and to determine the role of UV damage repair mechanisms.

Considering the high level of interest in the potential use of pulsed light, a greater understanding is required of inactivation and recovery mechanisms and their implications for the evaluation and optimization of these processes.

Resonance Initiated Field Effects

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Traditional laboratory techniques utilize high peak power, short duration, pulsed fields, to produce non thermal effects on micro-organisms. Pulsed fields, whether RF, electrical, or magnetic, are generally applied in a single burst with a corresponding non selective, and often detrimental, effect on cell viability. Investigations have been made into a method using resonance to produce similar non thermal, and cell type specific destructive effects, on micro-organisms.

All objects whether animate or inanimate, possess a natural period of vibration, which is also known as an objects Resonant Frequency. A pulsed; Electrical, Magnetic, or RF field may be used as a non thermal method of generating resonance initiated effects. Effects which include increased cell wall permeability and physiologic response. Resonance Initiated Field Effects (RIFE), significantly reduce applied power levels, and can negate unwanted, or detrimental, effects on non resonant cells and tissues. RIFE is induced through the repetitive usage of a small amplitude impulse of the same frequency as the natural vibration rate of the object. Such precisely timed impulses can produce large amplitude vibrations in the resonant object. When applied to a particular micro organism, a common effect of such resonance is outright destruction of the organism. The frequency that produces this effect is known as the "Mortal Oscillatory Rate," or MOR.

Investigative emphasis has been placed upon the determination of both organism and cell type specific, resonance's, or MOR's, using pulse repetition rates of <20kHz. RIFE depends upon exposure times measured in minutes, with application of many tens of thousands, to millions of resonant impulses.

There are several methods to determine a cells resonant frequency. The fundamental method utilizes the application of full positive or negative offset square waves to a sample set between two electrodes attached to a microscope slide. Spacing of the electrodes, voltage, and current lev-

els, all are independent influences, and must be considered in the determination of a particular cells resonant frequency.

Studies of RIFE using transmitted fields emitted by a pulsed, RF excited (27.125 MHz), enclosed gas plasma, offered unique challenges, and required adaptations to produce reliable correlations to non transmitted fields. Microscopes, slides, petri dishes, and test tubes, must be converted into antennas possessing a resonant frequency equal to the carrier frequency of the transmitter.

A theoretical method has been developed which may be useful in the determination of a particular organisms resonance frequency. This theory relies upon conversion of the number of base pairs in a strand of DNA, or particular gene, into a wave length, from which a frequency may be calculated. One may be able to use this frequency, or a sub harmonic of the frequency, to resonate a particular strand of DNA or gene. This process is patent pending, and is discussed with the consent of the inventor [1].

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High Intensity, Nanosecond Pulsed Electric Fields (nsPEF) Induce Apoptosis In Vitro And In Vivo And Inhibit Tumor Growth In Vivo

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Electromanipulation of biological cells, such as electroporation, has been studied for years, but the effects of nanosecond pulses on biological cells have not been tested until now. We analyzed the effects of high intensity (≤ 300 kV/cm), nanosecond (10-300 nsec) pulsed electric fields (nsPEF) on human and mouse cells *in vitro* and mouse tumors *in vivo*. Compared to electroporation pulses, nsPEF have durations two to six orders of magnitude shorter, electric fields hundred to thousand times greater, and energy densities five to ten-fold lower.

Objectives:

These studies were designed to determine the effects of nsPEF with different pulse durations on membrane pore formation, subcellular structures and functions, and cell survival. We hypothesize that nsPEF have minimal effects on plasma membrane poration, but induce apoptosis, in part, by targeting mitochondria *in vitro*, which provides a means to induce apoptosis and tumor regression *in vivo*.

Methods:

Human HL-60 and Jurkat cells, mouse fibrosarcoma B10.2 cells, and fibrosarcoma tumors *in vivo* were exposed to nsPEF and analyzed for apoptosis markers by flow cytometry, biochemical assays, and immunoblot analysis. Apoptosis markers included intact cell membrane, annexin V-FITC binding, caspase activation, and changes in cell size and density/granularity. Caspase was also analyzed by enzymatic assay and cytochrome c was identified by immunoblot analysis. Apoptosis was determined in tumors by a TUNEL-based method and tumor growth was determined by tumor size and weight.

Results:

As pulse duration decreased, transient membrane poration and pore size were also decreased. nsPEF-induced effects at the membrane and on cell function were distinctly different than electroporation. nsPEF induced apoptosis (programmed cell-death) in cells *in vitro*. Changes in cell size and density were caspase-independent, while annexin V-FITC binding was caspase-dependent. nsPEF induced cytochrome c release into the cytoplasm, suggesting that nsPEF targeted the mitochondria, well characterized initiators of apoptosis. nsPEF also induced apoptosis in mouse tumors *ex vivo* and *in vivo*, determined by caspase activation and DNA fragmentation, and decreased tumor growth rate and weight compared to controls.

Conclusions:

These studies support the hypothesis that nsPEF bypass the cell membrane and target subcellular structures [1]. Mitochondria appear to "sense" nsPEF and induce apoptosis similar to other agents that induce apoptosis by mitochondrial-dependent mechanisms. Applications for nsPEF include the selective targeting of subcellular structures and selective deletion of aberrant cells or tissues such as tumors without inflammation.

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Investigation of Bactericidal Properties of Water Treated by Electric Discharge of Low Energy

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A number of properties were detected in the course of investigation of bactericidal properties of water treated by pulse-periodical electric discharge with low energy in the pulse. They were called biological activity of water (BAW). One of manifestations of BAW is damaging action of treated water on micro-organisms. For example water damages *Daphnia*, bacteria *E. coli*, etc. Water retains its biological activity for a long period of time (more than 1 year). Physical or more exactly physical-chemical mechanism is not understood. Moreover the effect of biological active water while use it as drinkable water is not known. A number of investigations was carried out in order to study these problems. Their results are represented in the paper. The description of the installation for production of biological active water and the results of the treated water effect on micro-organisms are represented.

EMP Induces Apoptosis in Human Lung Carcinoma Cell Line A549

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Objective:

Observe apoptosis changes after being irradiated by EMP (electromagnetic pulses, EMP) to explore the possible injury mechanism in an human lung carcinoma cell line A549.

Materials and methods:

- 1.1 EMP simulator The EMP simulator was designed and established by the National University of Defense Technology . (electric field intensity is 60 kV/m, with 20 nsec rise time and 25 μ s pulse width).
- 1.2 Cell Lines and Culture Conditions. A human lung carcinoma cell line A549 was obtained from Shanghai Cell Biology Institute, Chinese Academy of Sciences. Cells were cultivated in RPMI 1640(Gibco) containing 10% calf serum (heat inactivated) at 37°C in an atmosphere containing 5% CO₂, 95% air and with a relative humidity of 98%. Cells are irradiated by EMP when the final cell concentrations are 104-5/flask. No treatment is given to the control groups. A total of 106cells were harvested at different times (0 hr, 1 hr, 6 hr, 12 hr, 24 hr, 48 hr) following irradiation and mixed with an equal volume of 0.4% trypan blue solution for 5 minutes. Stained and unstained cells were counted by a hemacytometer.
- 1.3 MTT Measurements. Cells were seeded in six 96 well culture plates at 104 cells/per well in 0.2 ml growth medium and incubated at 37°C for 24hrs, 1-6 columns were covered with double-layers of tinfoil as control columns. We then performed MTT stain at different times (0 hr, 1 hr, 6 hr, 12 hr, 24 hr, 48 hr) with BIO-ROD550 following irradiation.
- 1.4 Flow cytometer examination. Cells were trypsinized and suspended in PBS at 106cell/ml, washed with ice cold PBS and injected into cold(-20°C) 70% ethanol, which was kept overnight at 4°C. Cells were rinsed with PBS and stained with 50 μ g/ml PI PBS solution containing 0.1% triton-100,0.1 mM EDTA and 100U/ml RNase, incubated in the dark at room temperature for 30 minutes, then washed PI with

PBS, determined on flow cytometer(Facscalibure, Becton-Dickison, San Jose, CA) within 1 hour. All data were collected, stored and analyzed by Cellquest version 1.1.2 software(B-D).

1.5 Culture Cells on the Coverslips of 6 well Plates The cells were seeded on the 6 well plates with 4 coverslips for each well and incubated at 37°C until the growing cells covered about 50% area of one coverslip. The coverslips were fixed with 100% acetone at different time (0hr, 1hr, 6hr, 12hr, 24hr, 48hr) following irradiating, and stored at 4°C for immunohistochemical method examination.

1.6 Examination of Bcl-2 and P53 protein The immunohistochemical SP method was used to determine the protein bcl-2, p53. Bcl-2 and p53 positive stained cells were analyzed with CMIAS- II image analysis system at a magnification 400. All data was analyzed by SPSS 8.0 software.

Results:

EMP inhibited lung carcinoma cell line A549 proliferation and increased the nonadherent cells; The value of MTT decreased at immediate, 1 hr, and 6 hr after irradiating when compared with the controls($P<0.05$); The highest apoptosis ratios were determined by a flow cytometer at 6 hr after radiation. Down-regulation of Bcl-2 and up-regulation of P53 were found to be induced by EMP($P<0.05$).

Conclusion:

EMP promoted apoptosis of the human lung carcinoma line A549; EMP could also down-regulate Bcl-2 level and up-regulate P53 level in lung carcinoma line A549. The Bcl-2 and the P53 gene may take part in the process of apoptosis.

Pulsed Current Density Distribution Inside of Tissues Under Medical Treatment Defined by Numerical Analysis

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As described earlier in an Electromed'99 paper, it is important to have a biologically based recommendation for application of pulsed current for the treatment of neurological complaints. The known information in this area is obtained mainly from the study of self-contained samples of tissue, meanwhile in vivo the mutual influence of different kinds of tissue on the current density distribution must be taken into account. While the electrophysical and electrophysiological properties of different anatomical elements of human body cannot be considered as catalog readings, some useful information with respect to pulsed current density distribution can be obtained using the characteristics of similar contacted tissues, varying some of the data during calculations to account for the uncertainty.

The main purpose of such calculations is to study and to display, if possible, the probability of an unpredicted concentration of pulsed current in the separate layers of a multi-component area under conditions of pulsed electrical therapy. The author was only able to use a 2D simulation program, so results are obtained only for cross sections of anatomical structures. The presence of objects of different electrophysical properties such as skin layer, muscle tissue, bone tissue, blood-vessels and nerves were taken into consideration. A model of thorax cross section and limb section was used. The electroconductivity of each kind of tissue was accepted as a homogeneous value, although in the software program *QuickField* (Tera Analysis, USA) electroconductivity was accounted for as a tensor.

The resulting picture of current density distribution is depends strongly on the correlation of properties for the different kinds of tissue and, in addition, on the pulsed current parameters, but sometimes the latter can be considered as a standard factor. Pulsed current parameters are chosen from a desirable physiological action, but detailed analysis is able to correct some of the applied pulses. For example, one may optimize the pulse duration,

the number of pulses in a series and amplitude variation when applied to a specific area under treatment or specific complaints. When it is necessary to avoid pulsed current penetration into vital organs, the front of the pulses can be shorted and the pulse repetition increased. The living tissues, undoubtedly, suffer some after-effect from the current passing, but the numerical model, unfortunately, could not account for this process.

The some problems of software application to the simulation of pulsed processes were overcome at the work under discussion. The mentioned program *QuickField* is suitable for the analysis of pulsed processes only at the representation in view of steady-state ones. At the same time, the program *PDEase2D* (Macsyma Corp., USA) enable to simulate unstable process. Author has a pleasant duty to thank Sergey Konev (Kyiv Polytechnic) for assistance in use of *PDEase2D*. The model of time-dependent 2D electromagnetic field is implemented also in the FEM package, created in Kyiv Polytechnic. Author thanks Dr. Yuri Vaskovsky of Electromechanics Department for the assistance in this package made available for the solution of tasks in this work.

Pulsed Magnetic Field Influences on Blood Flow and Thrombosis Prevention

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A pulsed magnetic field of relatively low intensity can be used for the treatment of the consequences of such diseases as inflammation of blood-vessels to reduce damage from thrombosis. The first information about the possible interaction between flowing blood and magnetic fields was from an experiment run by Prof. V. Mitkevitch (Russia) in 1905, when he demonstrated that a magnetic arrow deviates in the presence of blood (described in the published collection of his scientific papers). The procedure of magnetic treatment has typically been applied to limbs using two circular coils supplied by pulsed current with a delay of the pulse in one coil relative to the other. The influence of a magnetic field on the flow of blood is able to be discerned in two ways: 1) on the molecular level, where the weak magnetic properties of blood interact with the magnetic field to cause aggregation of red blood cells more difficult; 2) on the macroscopic level, due to a possible acceleration or braking of red blood cell flow and the resulting speed of the blood flow under the action of magnetic forces. Field applications must be organized by such that both influences could lead to a reduction in the probability of blood clot creation. Analysis of magnetic field influences is obscured by the unclear role of small peripheral vessels.

Magnetic therapy has entered into medical practice in spite of the fact that the mechanism of its influence is not quite clear. We have attempted to use mathematic simulation to study the interaction of blood and magnetic fields. The first problem consists of building a model of blood as a non-magnetic fluid (emulsion) containing blood cells which possess weak magnetic properties. The preliminary part of the work includes a description of the collective motion of red cells inside a viscous fluid under the action of magnetic forces and a description of the individual motion of each red cell. The type of motion caused by the applied pulsed magnetic field should help to explain the role and contribution of microscopic and macroscopic factors in the interaction between blood and the field. Besides viscous friction,

the fluid model must also include the forces that cause aggregation of red cells. An approximate theory would involve using a hydrodynamic approach and use the theory of capillary processes. The main part of this work includes the analysis of red cell motion and aggregation using numerical solutions of equations. The method of pulsed field application is varied in the computations. Variation is assumed for both the time parameters of the pulses and of the mutual arrangement of coils.

The author hopes that the results of this analysis can be useful for better understanding of the influence of pulsed magnetic fields on the behavior of red cells and flowing of blood in blood-vessels, leading to the substitution of magnetic methods in the prevention of blood clots creation.

Effects of Microsecond and of Submicrosecond Electrical Pulses on Biological Cells

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An electrical model of a biological cell predicts that reducing the duration of high voltage pulses, to values at and below the charging time constant for the outer membrane, allows us to increasingly affect intracellular structures [1]. At the same time, the outer membrane effects, such as electroporation, decrease. In order to study the transitions from outer membrane effects to intracellular effects we have exposed Jurkat cells to electrical pulses of 100 μ s, 10 μ s, and 300 ns duration. The cells are placed between two plane-parallel stainless steel electrodes, 300 μ m apart, and immersed in a 1.5 μ m fluorescent dye, propidium iodide (PI). The uptake of the dye in response to the electrical pulses is observed with a microscope in real time. For the long pulses, the electric field between the electrodes was generated by means of a capacitive discharge circuit with a MOSFET as switch. With an applied voltage of 200 V, the electric field in the suspension was 6 kV/cm. These are pulse parameters similar to that used for electroporation of human erythrocytes [2]. A cable discharge, with a spark gap as switch, provided the 300 ns pulses. The voltage at the electrodes was 2 kV, corresponding to an electric field of 60 kV/cm. Upon application of the pulse the temporal development of the uptake of the dye by the cells was recorded by a low-light CCD camera with a temporal resolution of 10 ms. Fluorescence of PI is only observed after it passes the cell and combines with nucleic acid. Under electroporation conditions, immediate uptake of dye was observed; for the 300 ns pulse dye uptake was delayed by as long as 100 seconds. The immediate uptake of dye after long electrical pulses indicates that the surface cell membranes were opened as a direct consequence of the electric pulse application. This is expected under conditions where electroporation occurs [3]. The

delayed opening observed for submicrosecond pulses suggests that the opening of the outer membrane is a secondary effect, triggered by, rather than directly caused by the short pulse. A possible explanation is that the short pulses initiate apoptosis [4], which in turn causes delayed structural changes of the outer membrane. Independent of the processes, which ultimately cause the delayed membrane opening due to short pulse application, the results of the study demonstrate clearly the transition from classical electroporation to more complex changes at the surface membrane when shorter electrical pulses are applied.

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Small Size Solid State Nano and Picosecond Pulsers Using Fast Ionization Devices

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A new class of the superpower semiconductor switches of the closing type with a turn on time of less than 100 ps have been developed. These switches were called fast ionization switches (FID). The usage of FID allowed the development of a series of the small size pulsers, that have pulse widths from 100 ps to 100 ns, with pulse repetition frequencies up to several hundred kilohertz and peak power up to 1 MW. The pulsers have a PRF (pulse repetition frequency) of up to several kilohertz and can have peak power up to 1 GW. Pulsers with an amplitude of 10 kV and pulse widths from 100-500 ps typically occupy a volume of several tens of cubic centimeters. Pulsers with amplitude up to 100 kV usually have dimensions on the order of 200 x 300 x 300 mm.

A series of pulsers capable of generating 200-300 ps pulses with several hundred kiloamperes amplitude at several Ohm loads were developed. Pulsers that are able to operate with nonlinear loads, e.g. gas discharge have also been developed.

A Solid State Pulsed Power System for Pulsed Electric Field (PEF) Food Sterilization

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In 2000, Diversified Technologies, Inc. (DTI) of Bedford, MA designed, built, and installed the first commercial scale PEF system at Ohio State University's Department of Food Technology. This PEF system is part of a new food sterilization system assembled by a DoD sponsored, university-directed industry consortium. The purpose of the program is to research the use of high voltage pulsed power to sterilize liquid foods such as juices. The process, called Pulsed Electric Field processing (PEF), maintains the fresh taste of foods so often lost in heat pasteurization because it is non-thermal.

This solid state pulsed power system provides bipolar 65 KV, 600 A pulses at 75 kW average power into four PEF treatment chambers. It consists of two fully independent switching power supplies and solid state switches - one at positive voltage, and the other at negative voltage. This provides a very high level of pulse flexibility to the OSU researchers as they optimize PEF treatment parameters across a wide range of liquid foods.

In this paper we will describe the architecture of the pulsed power system and the application and benefits of solid state high voltage systems to food sterilization. Operational results and status may also be presented.

The Effect of Nanosecond Superbroadband Electromagnetic Radiation on Xenogeneic Erythrocytes

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Experiments were made to investigate the effect of short-pulse superbroadband electromagnetic radiation on biological objects. An insulated rod antenna excited by a high-current electron beam ($E \sim 0.5$ to 1.0 MeV, $I \sim 4$ to 10 kA, $\tau \approx 15$ ns) served as a radiation source. The objects to be irradiated, i.e., erythrocytes of both donors and diabetics, were put in the regions with field strengths varying from 100 to 1000 kV/cm. The effect of radiation on the lifetime and shape of erythrocytes, on the permeability of erythrocyte membranes for the penetrating nonelectrolyte (1M glycerin) and the state of intracorpuseular hemoglobin was investigated by the methods of small-angle light scattering, UV spectrometry and phase-contrast microscopy.

Small portions of blood (5 mL) were exposed to different numbers of pulses (1, 5, 10) with 90 to 100 s intervals between the pulses at temperatures between 16 and 20°C . Their characteristics were examined in 3 to 5 hours after irradiation.

The microscope studies have revealed no differences in the shape of irradiated and control cells. The coefficient of erythrocyte permeability for glycerin molecules, determined from the time of 50% hemolysis in the 1M water solution of glycerin at 37°C , increased or decreased depending on the number of pulses at a field strength of ~ 1000 V/cm. Most likely the diversity in the permeability coefficient values is due to both

the individual characteristics of donor/patient blood and the composition of the medium where the cells had been at the time of irradiation (plasma or physiological salt solution). As compared to the reference specimens, irradiation has increased by 30% the hemolysis of erythrocytes only in the donor blood having a rather high content of methemoglobin, the degree of hemolysis being independent of the number of pulses.

The experiments have revealed that the biological effect manifests itself at higher field strengths as compared to a narrow-band microsecond microwave radiation.

Response of Adherent Cells to Diamagnetic Torque Force

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Recent studies reported a new approach to manipulate cells in a non-contact condition using magnetically aligned protein polymers. Tranquillo et al. [1] reported the orientation of smooth muscle cells, which were embedded on magnetically oriented collagen gel. Also, Torbet reported the orientation of endothelial cells on magnetically oriented fibrin fibers [2]. On the other hand in 1993, Higashi et al. reported the magnetic orientation of red blood cells and platelets, which were floating in an aqueous solution [3]. In March 1997, we were the first to observe a novel phenomenon of adherent smooth muscle cells directing their long axis along static high magnetic fields of 8 tesla, as reported in 1999 [4].

Rat smooth muscle cell (A7r5) was cultured in a polystyrene flask for two weeks in magnetic fields of up to 8 T. The pilot experiments started with a low cell density, and reached a confluent in flask after 17 days of incubation under 8 tesla static magnetic field in a medium (D-MEM, 10% FBS) at 37 degrees centigrade. Control cells formed a micro domain of uni-axially oriented cells, however, all of the micro domains in a flask oriented randomly. Occasionally, a chain of micro domains formed a "vortex" pattern. In the magnetic field exposed flask, the pattern of micro domains changed to a "stream" pattern, directed to the magnetic field direction. The cell domain alignment did not occur prior to 15 days, however, the adherent smooth muscle cell's assembly directed its long axis along static high magnetic fields in the 17th day. The results indicated that the alignment manifest between the 16th day and 17th day when the cell density was confluence or pre-confluence. The all of the cells in the flask were directed along its long axis toward the direction of the magnetic field with an angle variance of 30 degree. No cells were directed normal to the magnetic field direction.

In the present study, the experiments with a 14 tesla superconducting

magnet exhibited a rapid uniaxial alignment of cell assembly domains. The smooth muscle cell domain aligned within 40 hours under 14 tesla, and increasing initial cell density resulted in complete uniaxial alignment, which resembled the magnetic orientation of collagen and fibrin fibers. The possible origin of the observed phenomenon was a diamagnetic anisotropy of smooth muscle cell, which has a rod-like shape adhering on a substrate. Higashi et al. had reported the magnetic orientation of floating cells, such as blood platelets and red blood cells. It was, however, more difficult for adherent cells themselves to orient even with a diamagnetic torque energy of 10 tesla. The observed magnetically ordered cell-assembly-domains structure was exhibited when the total magnetic anisotropic energy of a cell-assembly-domain exceeded the background thermal agitation and the friction of culture dish's surface.

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Using High-Current Nanosecond Electron Beams To Sterilize Liquid Eggs

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The liquid eggs (or melange) are pasteurized for 40 seconds at a temperature of 60 °C. In this case, 98-99% vegetative microflora were killed. However, this treatment has significant drawbacks. Much energy is consumed for the pasteurization process. Moreover, pathogenic micro-organisms remain alive. The radiation sterilization represents an alternative approach. A drawback of the radiation sterilization is that it is hazardous to the attending personnel. However, the hazard can be considerably reduced by optimizing the radiation source. This study deals with the elaboration of principles of a technology for sterilizing the melange and the development of a model installation for realizing the technology. In accordance with the proposed technological scheme, the melange is treated in a chosen atmosphere (argon).

Consequently, the effect of air radiolysis products on the raw material is nearly eliminated. The irradiation chamber is formed by the output foil of the electron accelerator and a stainless-steel flange, which are spaced 1 mm (considering the penetration depth of 0.5-MeV electrons). Vacuum-produced chambers of the foil act as flow separators. As a result, the melange is mixed efficiently and is irradiated more uniformly in depth.

A sample, which was infected beforehand with *Salmonella* having the concentration of 100 l/mL, was exposed to different absorbed doses. The maximum absorbed dose on the chamber surface did not exceed 50 kGy. Later measurements were made using smaller absorbed doses. The accelerator frequency was up to 30 Hz. It was assigned considering the required absorbed dose for the melange moving in the chamber at a speed of up to 10 cm/s, which was preset by the argon pressure.

Microbiological and properties conformity tests were performed at laboratories using standard techniques.

The tests showed that the melange was sterile after exposure to an absorbed dose of 14 kGy. The consumer properties of the melange did not change up to 50 kGy. It was found that the pH value of the melange decreased slightly with the absorbed dose level and the mass fraction of the protein increased. However, changes in the liquid egg composition were insignificant and approached the measurement error.

Using Nanosecond High Electric Field Pulses for Inactivation of Micro-organisms

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We report on the first experiments to use nanosecond high electric field pulses (HELP) for inactivation of micro-organisms. We used the high voltage pulse generator created for the URT-0,2 accelerator [1]. It has the following operational characteristics: voltage up to 200 kV; a FWHM pulse duration from 15 to 34 ns; operating frequency up to 250 Hz.

Special design treatment chamber was created. This chamber consisted of two stainless steel cylinders, which were squeezed into a sample within the dielectric tube. The electrode area was 7 cm², the gap was 1 cm.

The effect of field strength, pulse energy, pulse amount and repetition rate on the inactivation of *E. coli* and *Staphylococcus aureus* was investigated. The initial microbial count of both culture was from 10⁴ to 10⁸ 1/mL.

The pulse associated with full sterilization was found when the field strength was 100 kV/cm. From our experiments it was shown that there is a difference in HELP impact on various kind of microorganism.

The results show that repetitively HELP generators are highly promising and are worth considering for use in commercial sterilization and pasteurization applications.

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**Pulsed Signal Therapy for Osteoarthritis:
Clinical Trial Results in over 100,000 Patients
with Supportive *In Vitro* Studies**

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Bone atrophies in the absence of physical pressure such as weightlessness during space travel or immobilization in a cast. Conversely, regular exercise increases the density and strength of weight bearing bones. Pulsed Signal Therapy (PST) is the result of three decades of research designed to characterize the piezoelectric signal that initiates these regenerative activities by creating a streaming potential in the extracellular matrix when bone is placed under a load. Although the optimal potential and transmission of this rejuvenative signal is impaired in osteoarthritis, PST passively duplicates this streaming potential and its restorative rewards in affected joints even though they are at rest. PST is administered via a magnetic field generator that emits a proprietary pulsed electromagnetic field by means of a connected ring-shaped applicator coil. Applicator devices with different coil sizes have been developed to treat peripheral joints (knees, shoulders and wrists or hands and elbows), the axial spine (cervical, thoracic and lumbar vertebral bodies), tinnitus and dental disorders, and for specific veterinary applications. The joint to be treated is placed inside the applicator coil and exposed to physiological frequency/amplitude combinations that are switched automatically so that an induction phase takes place during the first 10 minutes followed by a combination of therapeutic pulsed stimuli for the remaining 50 minutes. PST is usually administered for an hour a day for nine consecutive days interrupted only by a weekend. No further treatment is usually required. As will be illustrated, the ability of PST to reduce pain and improve functional mobility and range of motion in osteoarthritis has been confirmed in double blind and other clinical trials in over 100,000 patients. Treatment is non-invasive, not associated with any adverse side effects and long term follow-up studies confirm sustained pain relief, improved mo-

bility and complete safety. Over 50,000 patients were prescribed PST in 1999 by over 1,000 physicians at over 300 hospitals and medical clinics in sixteen countries where it is usually reimbursed by fiscal intermediaries and governmental agencies because of its proven record of cost effectiveness and safety. PST devices are currently approved in the U.S. only for veterinary applications. Pulsed Signal Therapy differs from other electromagnetic approaches that are often in the public domain because of its specific amplitude, frequency and repetition parameters. PST's patented signal (pulsed DC magnetic field 0.28 W., max. 20 gauss; 5-24 Hz; quasi-rectangular wave form) is the only electromagnetic stimulus with documented, long term, multi-center clinical study proof of efficacy in large numbers of patients and safety in rigorously controlled clinical trials. In sharp contrast to other devices that make similar claims, the proposed mechanisms of action of PST are also supported by extensive basic science *in vitro* studies demonstrating increased chondrocyte production and proteoglycan synthesis in human chondrocyte cultures.

Pulsed Power Sources for Bio-Medical Applications

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Recent advances in the generation of ultra-short electrical pulses at very high power levels have provided new opportunities in the field of bioelectrics. This paper discusses several technologies currently available that deliver ultrashort pulses. Included are generators that can deliver voltage pulses ranging from a few kV to several hundred kV, with risetimes as short as several hundred pico-seconds. Additional discussions will include systems designed to deliver multiple pulses from single sources as well as multiple sources, and pulse-shaping generators.

Fluorescence Assessment of Bacterial Respiration after Treatment with Pulsed Electric Fields

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There is considerable interest in the use of pulsed electric field (PEF) technology as an alternative or complementary method for the treatment of foodstuffs. The optimization of this technology will however require a fuller understanding of the effects of PEF on microbial cells and particularly on the extent of cellular damage caused by PEF treatment. To this end, experiments have been undertaken to determine the effects of PEF on the functional electron transport activity (i.e., respiratory activity) of microbial cells.

Test organisms included the potential bacterial pathogens *Listeria monocytogenes*, *Bacillus cereus* and *E. coli* were grown overnight in tryptone soya yeast extract (TSYE) broth. Pre-determined cell populations were suspended in 0.1% peptone water and were heated at 56°C or treated with high intensity pulsed electric fields (30 kV/cm with a pulse duration of 500 ns). Treated microbial populations were then enumerated by spiral plating samples onto tryptone soya yeast extract agar (direct viable counts or DVC), and by determining the number of respiring cells by staining with the novel redox sensitive dye 5-cyano-2,3-ditolyl tetrazolium chloride (CTC). Use of image analysis and counterstaining CTC with 4,6-diamidino-2-phenylindole (DAPI) allowed simultaneous determinations of total cell numbers (blue), and those bacteria that were actively respiring (red). While all the bacteria in the treated samples stained blue by DAPI, only respiring cells capable of the intracellular reduction of CTC to the insoluble fluorescent formazan crystal (i.e., indicative of cellular respiration) strained red.

The results have demonstrated, under the experimental conditions used,

that the application of heat at 56°C and the application of PEF resulted in a similar lethality, with the test bacterial populations being reduced by approximately 5 log orders as determined by direct viable counts (DVC) on TSYE agar. However, upon microscopic examination of the heat and PEF treated populations, using CTC-DAPI double staining, it was found that both treatments produced sub-populations of bacteria that had functional electron transport systems (ie were respiring) but were incapable of growth or cell doubling on nutritious TSYE agar. The sub-population following heat-treatment was significantly greater than sub-population that survived PEF treatment. This result illustrates a potential benefit associated with PEF treatment of foodborne bacterial pathogens as PEF treatment produces significantly lower numbers of non-replicating bacteria that possess residual respiratory activity.

Generation of Picosecond, High Peak-Power Infrared Pulses using a Compact Free Electron Laser

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The University of Maryland is currently constructing a compact free-electron laser (FEL) designed to produce infrared and far-infrared pulses at very-high peak power. FELs have become important light sources at wavelengths where other sources are weak. Of particular interest is the mid to far IR region where there is a lack of ultra-short pulse, high-peak power, coherent sources. The Maryland Infrared FEL (MIRFEL) is a table-top device capable of producing trains of picosecond pulses with multi-megawatt peak power. These characteristics make it useful for many biological applications. This paper discusses the design of the laser and its expected output.

Processing of Sugar Beets with Pulsed Electric Fields

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The treatment of biological cells with strong pulsed electric fields can lead to irreversible formation of large pores in the cell membrane and thus destroy the cell and give access to its content. This well-known process of electroporation has been successfully applied to the inactivation of bacteria in many laboratories. However, only few efforts have been made to utilize the technique on a large industrial scale for the production of nourishment from food plants. We have built the mobile test device KEA (Karlsruher Elektroporations Anlage) which consists of a 300 kV Marx generator operating at 20 Hz and delivering its pulses to a cylindrical reaction chamber with axially and azimuthally distributed electrodes. The reaction chamber has a large cross section, sufficient for the treatment of entire fruits in a continuous stream. KEA has been used in an experimental campaign at a sugar factory to demonstrate the advantages of electric pulse treatment compared with conventional techniques for the production of sugar from beets. Although the process had not yet been optimized it was found that appreciable energy savings are possible since the treated beets could be extracted at much lower temperatures with the same yield. To demonstrate the technical and economic feasibility on a large scale we plan to build a facility with a throughput of 80 t/h and plan to use it in the next seasonal campaign. Although the results are convincing, important details of the effect are not yet understood. Especially the mechanism of electroporation in a complete plant organism with walls between the individual cells needs further investigation. Therefore, a basic research program to address these questions is required.

DNA Damage Visualized by Comet Assay Following Nanosecond Pulsed Electric Field Applications to Eosinophils

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The past 20 years has seen the advent of kV/cm microsecond duration electric field technology applied to cells in order to induce formation of transient pores in cell surface membranes. This has allowed insertion of biomolecules into exposed cells and has evolved into an important biomedical technology (electroporation) with widespread applications. In an extension of this technology, our laboratories have used biochemical, morphologic and electron microscopic methods to examine whether kV/cm nanosecond pulsed electric field (nsPEF) applications to cells can achieve the predicted effects of pore formation and/or disruption of intracellular membranes. The biological model utilized human eosinophils, cells that contain large intracellular granules whose highly cationic contents are normally membrane bound but, when this membrane is damaged, can experimentally react with calcein to form highly fluorescent granules or "sparklers." The formation of "sparklers" gives a measurement of the amount of damage to the internal membrane of these structures. Using the same cell type, we have simultaneously examined cells for "sparkler" formation and, by the comet assay, determined whether nsPEF can induce DNA damage within the membrane of the cellular nucleus. nsPEF applications to eosinophils resulted in the formation of "sparklers" in an approximate dose dependent manner. We exposed cells to 1, 5, or 10 pulses for each of 0, 10, 60 or 300-nanosecond duration at 1.7J/cm² and 1, 5, or 10 pulses for 12ns duration at 6.8J/cm². The number of "sparkler" positive cells increased from none in the non-exposed cells to 75% in the 12ns 6.8J/cm² exposed cells showing that these doses induce internal membrane damage. DNA damage was quantified as the length of the comet

tail produced following alkaline electrophoresis of single cells embedded in agar measured as tail length in pixels. Although there was a clear distinction between the comet tail length obtained in the controls versus nsPEF exposed cells, there was no clear trend in tail length with increasing pulse train size, indicating that the effects of nsPEF on DNA at these exposures appears to be an "all or nothing response." Our experiments also distinguished differences in tail length between two different cell types exposed at the same time. Starting cell preparations contained a mixed population consisting of 40% eosinophils and 56% neutrophils. After nsPEF applications, we could identify one population of bright cells with short tails, and another population of less bright cells with a longer tail, in approximately the same proportions as obtained in the original cell isolation, suggesting differential sensitivity of nuclear DNA to damage in these two cell types. Overall, these results demonstrate that selected nsPEF conditions can cause disruption of internal cellular membranes without the disruption of the surface membrane and that exposure to these conditions can cause DNA damage. Refinement of nsPEF application conditions may allow intracellular membrane and DNA effects to be achieved selectively in living cells based upon cell type.

Biological Effects of Electron Beam at the Membrane Level

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Sources of electrons differing in characteristics are presently extensively used in the medical practice. In view of this it seems of importance to identify the molecular processes contributing to the biological effect of an electron beam. A number of such processes are proved to occur in cellular membranes. To gain further insight into the nature of membrane modification on irradiation we examined the influence of 5 MeV electrons on a variety of biophysical and biochemical parameters reflecting alterations in the structural and functional state of membrane components. These include: electrophoretic mobility of cells (erythrocytes and thymocytes), intrinsic fluorescence of membrane proteins, spectral characteristics of fluorescent probes, the level of lipid peroxidation and the activity of membrane-bound enzymes (ATPases of erythrocyte and oxidoreductases of microsomal membranes).

Irradiation to doses of 10^{-10^3} Gy was found to cause decreasing EFM of isolates erythrocytes and thymocytes, originating, presumably from the changes in the spatial charge distribution in the oligosaccharide membrane layer due to reduced microviscosity of the lipid phase and increased mobility of the protein molecules.

Examination of the membrane-associating properties of the anionic (1,8-anilinonaphthalene sulfonic acid) and cationic (4-n-dimethylaminostyryl)-1-methylpyridine fluorescent probes revealed radiation-induced alterations in the structure of lipid bilayer manifested themselves in the increasing negative electrostatic potential at the lipid-water interface.

Studies of the quenching of the intrinsic protein fluorescence provided evidence for the rising structural rigidity of the erythrocyte and thymocyte membrane proteins on irradiation, the effect being related to the development of lipid peroxidation.

The observed inhibition of the erythrocyte Ca^{2+} and Mg^{2+} -ATPases and microsomal oxidoreductases was shown to be mediated by the modification of the lipid membrane phase by the products of water radiolysis.

Physical mechanisms of interaction of high energy electrons with the membrane components are discussed in terms of the modern theories of radiobiological effects.

The mechanism of local thermoacoustic effect of high energy electrons on the biological environment was theoretically investigated and the typical sizes of the area of local influence were shown to be comparable with the size of membrane structures.

Nanosecond Pulsed Electric Fields Induce Apoptosis in 3T3-L1 Cells: A Comparison of Apoptosis Induction by Fas

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Background:

We have demonstrated that high intensity (kilovolt/centimeter, kV/cm), low energy (joule/cubic centimeter, J/cc), ultra-short duration (nanoseconds) pulsed electric fields (nsPEF) induce apoptosis in tumor cells and tissues. Because little is known about the roles, mechanisms, and pathways for apoptosis in fat cells, or about the role apoptosis plays in the control of fat cell development and homeostasis in the obese state, we investigated apoptosis in 3T3-L1 pre-adipocytes. We compared mechanisms of apoptosis in 3T3-L1 cells induced by nsPEF and anti-Fas antibody, a known apoptosis stimulus in this pre-adipocyte model.

Methods included flow cytometry with several fluorescent apoptosis markers; enzyme (caspase) assays, and immunoblot analyses for caspase-3, caspase-9 and cytochrome c. Three different assays for caspase activation were used to confirm apoptosis for each stimulus. These included a fluorogenic assay with DEVD-afc caspase3-like substrate; a fluorescent, cell permeable, irreversible inhibitor of active caspases; and immunoblot analysis with caspase3-specific antibodies.

Results indicate that as in Jurkat cells, nsPEF and anti-Fas induce apoptosis in 3T3-L1 cells as determined by Annexin-binding and caspase activation. However, longer pulses, higher electric fields, and/or more pulse repetitions were required to induce apoptosis in 3T3-L1 compared to Jurkat or HL-60 cells. For example, applying 300kV/cm to cells in suspension, Jurkat cell apoptosis was induced with three repetitive 10 nsec pulses while 3T3-L1 cells required ten repetitive 300 nsec pulses.

For a 300 nsec pulses, Jurkat cell apoptosis was induced with an electric field of ≤ 150 kV/cm while 3T3-L1 cells required 300kV/cm. Cytochrome c release in occurred in nsPEF treated 3T3-L1 pre-adipocytes and Jurkat cells, whereas no cytochrome c release occurred in anti-Fas treated cells.

Conclusions:

Nanosecond pulsed electric field treatment of Jurkat cells and 3T3-L1 pre-adipocytes induces apoptosis by a mitochondrial mechanism with release of cytochrome c. Compared to Jurkat and HL-60 cells, 3T3-L1 cells are more resistant to apoptosis induction by nsPEF. This is due, at least in part, to the larger size of 3T3-L1 cells, but the effects of membrane and cell cytosol constituents on thresholds for nsPEF-induced apoptosis have not been investigated. A characterization of the threshold for apoptosis induction among different cell types will provide valuable insight into the characteristics of nsPEF that couple to intracellular apoptosis mechanisms and will define the intracellular targets and how they sense nsPEF.

The Effect of Electromagnetic Pulse on Hippocampus LTP and Learning and Memory Ability in Rats

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Objective:

In this study, we observed the test of Y maze and hippocampal LTP to investigate the effect on the ability of learning and memory and hippocampal neuro-synaptic plasticity in rats irradiated by electromagnetic pulse (EMP).

Methods:

EMP simulator was designed and established by National University of Defense Technology(CHINA); DANTEC Neuromatic 2000M μ Nihon Kohden Electronic Stimulator SEN-320115; Wistar male rats were divided into two groups: EMP irradiated group and a control group. The electromagnetic pulse simulator which provides 2.5 pulses/min with a high electric field intensity (60 KV/m), and 20-nsec rise time and 25 μ s pulse wide. The hippocampal LTP was induced within 6 h (5 animals) and after 12 h (5 animals) following irradiation. 20 Wistar rats were divided into an EMP group and a control group for the test of Y maze. The learning ability is the correct rate of the first day's test, the memory ability is the correct rate of the second day's test after being irradiated.

Results:

Suppression in LTP($148.0\% \pm 4.9\%$) in hippocampal DG was observed ($P < 0.01$) in the EMP group within 6 h, and tended to return to control group($204.4\% \pm 2.9\%$) after 12 h. The spatial learning and memory ability in the EMP group were different from control group($P < 0.05$).

Conclusion:

The results imply that some changes may occur in the hippocampal neuronal morphology, functional and synaptic plasticity with EMP irradiation. Learning and memory ability may also be consequently adversely affected.

The Influence of the Expression of c-Fos in the Hippocampus by EMP

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Objective:

With observing the changes of expression of c-fos in hippocampus and dentate gyrus after being irradiated by EMP(electromagnetic pulses, EMP) to explore the possible injury mechanism in rat's brain.

Methods:

EMP simulator was designed and established by National University of Defense Technology. (Electric field intensity is 2000-60 KV/m, with 20-40nsec rise time and 25-30 μ s pulse wide). 80 Wistar rats(40 males and 40 females) were divided into irradiated groups(subdivided as groupA, groupB and groupC, with different electric field intensity 6x104V/m, 2x104V/m and C-6x103V/m irradiating respectively) and control group. The groupA, groupB and groupC were irradiated by 5 EMP within 2 minutes respectively, no treatment to control group, then the rats were killed at 1h, 6h, 12h, 24h and 48h separately. Immunohistochemical methods was used to investigate the expression of c-fos and stereological analysis for c-fos positive cells were performed by CMIAS- II image analysis system at a magnification 400.

Results:

The irradiated groups rats had higher number of c-fos neurons in hippocampus and dentate gyrus at 12-24h following irradiating by EMP. The number of c-fos cells is significant higher comparing with control group($p<0.05$).

Conclusion:

The EMP could affect the learning and memory ability of rats, and the significant high c-fos expression in hippocampus and gyrus maybe plays an important role in the injury of rats brain by EMP.

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Symposium Agenda

SUNDAY	
6:00 pm	Registration and Reception at the Renaissance Portsmouth Hotel
MONDAY	
8:45	1. <i>Foster</i> – What Are the Risks of Microwave Radiation
9:25	2. <i>Blank</i> – Electromagnetic Fields Accelerate Electron Transfer and Initiate Transcription
10:00	3. <i>Lai</i> – Effects of electromagnetic Fields on the Central Nervous System
10:40	4. <i>Tofani</i> – Anticancer Activity by Nonthermal Magnetic Fields
11:00	5. <i>Pakhomov</i> – Retaining of the Long Term Potentiation in Hippocampal Slices After High Peak Power
11:20	6. <i>Cho</i> – Electrotherapy of Wound Healing: Mechanisms of Action on Cell Structures and Function
1:30	7. <i>Joshi</i> – Modeling of Biological Cells Subjected to High Intensity Electrical Pulses
2:10	8. <i>Hassan</i> – Mapping Membrane Potential Perturbations of Chromaffin Cells Exposed to Electric Fields
3:00	9. <i>Rabussay</i> – Advances in Electroporation Therapy
3:40	10. <i>Buescher</i> – Nanosecond Pulsed Electric Field (nsPEF) Applications to Human Cells Results in Selective Intracellular Membrane Disruption
4:10	11. <i>Beebe</i> – High Intensity, Nanosecond Pulsed Electric Fields (nsPEF) Induce Apoptosis In Vitro And In Vivo And Inhibit Tumor Growth In Vivo
4:40	12. <i>Goodman</i> – Biomedical Application of Electromagnetic Fields for Cytoprotection And Gene Therapy

Symposium Agenda (continued)

TUESDAY

8:30	1. <i>Curry & McDonald</i> - Rapid Sterilization of Surfaces Using Advanced Photosensitizers and UV Light
9:00	2. <i>Kiel</i> - Directed Killing of Anthrax Spores by Microwave Induced Cavitation Via Specific Binding of Organic Semi-Conductor
9:30	3. <i>MacGregor</i> - The Influence of Pulsed Electric Field Profile on the Inactivation of Microorganisms
10:00	4. <i>Herrmann</i> - Biological and Chemical Decontamination Using Near Atmospheric Pressure Plasma Discharges

10:45	5. <i>Alexeff</i> - The Uniform Steady State Atmospheric DC Plasma
11:15	6. <i>Laroussi</i> - Biochemical Pathways on the Interaction of Non-Equilibrium Plasmas with Bacteria
11:45	7. <i>Akiyama</i> - Production of NO for Medical Applications using Pulsed Power Technology

1:30	8. <i>Roth & Sherman</i> - An Improved Plasma Reactor Configuration for Surface Treatment and Sterilization Based on the One Atmosphere Uniform Glow Discharge Plasma
2:00	9. <i>Kenning</i> - Plasma Treatment of Biological Materials for BioDetection
2:30	10. <i>Trompeter</i> - Reduction of Bacillus and Aspergillus Niger Spores Using Nonthermal Atmospheric Gas Discharges

WEDNESDAY

9:00	1. <i>Breakout Session</i> - Cellular and Systemic Effects, E.S. Buescher, Chair
9:00	2. <i>Breakout Session</i> - Sterilization, I. Alexeff and M. Laroussi, Chairs

Symposium Agenda (continued)

11:00	3. <i>Combined Sessions</i> - Chairs
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Social Program

A variety of social events are planned for the duration of the Symposium.

Sunday, May 20

Welcome reception at the Renaissance Portsmouth Hotel begins at 6:00 p.m.

Monday, May 21

The Symposium Banquet will be held Monday evening at the Chrysler Museum from 6:30 pm to 9:30 pm. The Chrysler Museum houses a collection of over 30,000 objects spanning nearly 4,000 years of Art History. Highlights include an internationally famous glass collection that includes holding of blown glass made by Tiffany Studios and outstanding Tiffany lamps. The fee is \$25 for this event.

Tuesday, May 22

A dinner cruise on the American Rover, a three-mast schooner, is from 6:30- 9:30 pm with boarding at 6:15. We will be boarding at the Waterside Hotel—across the water from the Renaissance Portsmouth Hotel. A ferry is available to get from the Renaissance Portsmouth Hotel to the Waterside Hotel. The cruise is available for an additional cost of \$30. Registration is optional and required at the time of conference registration.

Travel Grants for Graduate Students

The Whitaker Foundation provided ten travel awards of \$500 each for travel support to graduate students and young postdoctoral scientists to attend the symposium. Applicants for student travel grants were asked to send a cover letter asking to be considered for support, a short CV, and a letter of recommendation from their Department Chair indicating that the Department is committed to provide additional support for student travel. A short statement describing the need for support was also requested. Preference was given to those who were authors or co-authors of an accepted abstract or submitted an abstract with their application. The Organizing Committee made the award selections.

Special Issue of the IEEE Transactions on Plasma Science

**"Nonthermal Medical/Biological Treatments Using
Electromagnetic Fields and Ionized Gases"**

(Scheduled for August 2002)

Guest editors for the special issue include Mounir Laroussi (Old Dominion University), Robert Stark, (Old Dominion University), and Stephen Beebe (Eastern Virginia Medical School).

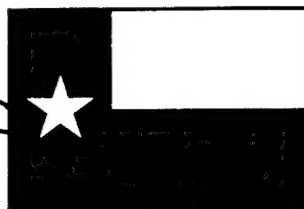
Recent advances in the generation of ultrashort high power electrical pulses have opened new venues in the field of bioelectrics. Electrical pulses of duration less than a billionth of a second but at voltages exceeding ten thousand volts allow one to explore and to utilize electrical interactions with biological cells without significant heating of the tissue. The high frequency components in the ultrashort pulses have been shown to provide an effective pathway to the interior of the cells. Pulsed, high power microwave and millimeter wave sources allow one to similarly explore and utilize non-linear processes on the molecular level, with the potential to some day selectively modify individual molecular structures, such as DNA.

Equally exciting is the growing field of research into the application of plasmas for chemical and biological sterilization and decontamination. A number of industrial and university research groups have already demonstrated the remarkable ability of relatively cold ionized gases to rapidly kill bacteria cells while avoiding the excessive heat and/or harsh chemicals associated with current conventional sterilization techniques. This new approach poses major advantage for both defense and commercial medical applications.

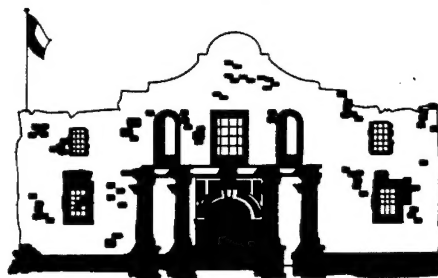
The intent of the TPS Special Issue is to provide a wider forum for this topic and to assemble papers addressing both the fundamental and applied aspects of nonthermal electromagnetic and plasma effects on biological cells. Contributions are solicited in, but not restricted to, the following areas:

-
- Electroporation of Cells and Tissues
 - Medical Applications of Electroporation
 - Pulsed Electric Fields for Debacterialization
 - Interaction of High Frequency Electromagnetic Fields with Biological Systems
 - Pulsed Microwave Induced Bioeffects
 - Biological Effects of Millimeter Waves
 - Air Plasma Sterilization of Surfaces and Materials
 - Bacterial Decontamination Using High Pressure Nonthermal Discharges

Please submit three copies of the manuscript, original figures, and a signed IEEE copyright form to one of the three guest editors whose addresses appear below. The deadline for submitting manuscripts is July 15, 2001. To ensure maximum speed in correspondence, please include telephone and FAX numbers and email address of the corresponding author. Further detailed instructions for authors may be found on the inside back cover of the IEEE Transactions on Plasma Science, and on the web: www.IEEE.org/pubs/authors.html.



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